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TITLE OF INVENTION
Novel FabH Enzyme Composition Capable of Binding to Said Enzyme and Methods of Use Thereof

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NOVEL FABH ENZYME, COMPOSITIONS CAPABLE OF BINDING TO SAID ENZYME AND METHODS OF USE THEREOF

Technical Field of the Invention

The invention relates to the identification of a novel enzyme active site and methods enabling the design and selection of inhibitors of that active site.

Background of the Invention

The pathway for the biosynthesis of saturated fatty acids is very similar in prokaryotes and eukaryotes. However, the organization of the biosynthetic apparatus is very different. Vertebrates possess a type I fatty acid synthase (FAS) in which all of the enzymatic activities are encoded on one multifunctional polypeptide, the mature protein being a homodimer. The acyl carrier protein (ACP) is an integral part of the complex. In contrast, in most bacterial and plant FASs (type II) each of the reactions are catalyzed by distinct monofunctional enzymes and the ACP is a discrete protein. Mycobacteria are unique in that they possess both type I and II FASs. There therefore appears to be considerable potential for selective inhibition of the bacterial systems by broad-spectrum antibacterial agents (Rock, C. & Cronan, J. 1996. *Biochimica et Biophysica Acta* 1302, 1-16; Jackowski, S. 1992. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*. Ed. J. Sutcliffe & N. Georgopapadakou. Chapman & Hall, New York; Jackowski, S. *et al.* (1989). *J. Biol. Chem.* 264, 7624-7629.)

The first step in the biosynthetic cycle is the condensation of malonyl-ACP with acetyl-CoA by FabH. Prior to this, malonyl-ACP is synthesized from ACP and malonyl-CoA by FabD, malonyl CoA:ACP transacylase. In subsequent rounds malonyl-ACP is condensed with the growing-chain acyl-ACP (FabB and FabF, synthases I and II respectively). The second step in the elongation cycle is ketoester reduction by NADPH-dependent β-ketoacyl-ACP reductase (FabG). Subsequent dehydration by β-hydroxyacyl-ACP dehydrase (either FabA or FabZ) leads to trans-2-enoyl-ACP which is in turn converted to acyl-ACP by enoyl-ACP reductase (FabI). Further rounds of this cycle, adding two carbon atoms per cycle, eventually lead to palmitoyl-ACP whereupon the cycle is stopped largely due to feedback inhibition of FabH and I by palmitoyl-ACP (Heath, et al, (1996), *J.Biol.Chem. 271*, 1833-1836).

Cerulenin and thiolactomycin are potent and selective inhibitors of bacterial fatty acid biosynthesis. Extensive work with these inhibitors has proved that this biosynthetic pathway is essential for bacterial viability. No marketed antibiotics are targeted against fatty acid biosynthesis, therefore it is unlikely that novel antibiotics would be rendered

inactive by known antibiotic resistance mechanisms. There is an unmet need for developing new classes of antibiotic compounds, such as those that target FabH.

FabH enzymes are of interest as potential targets for antibacterial agents.

There is a need in the art for novel FabH enzyme active sites and catalytic sequences to enable identification and structure-based design of inhibitors, which are useful in the treatment or prophylaxis of diseases, particularly diseases caused by bacteria which may share catalytic domains with those of the invention.

Summary of the Invention

In one aspect, the present invention provides a novel FabH enzyme active site crystalline form.

In another aspect, the present invention provides a novel FabH composition characterized by the catalytic residues Cys112, His244 and Asn274.

In still another aspect, the present invention provides a novel FabH composition characterized by the active site of 33 amino acid residues (including the catalytic residues).

In yet another aspect, the invention provides a method for identifying inhibitors of the compositions described above which methods involve the steps of: providing the coordinates of the structure of the invention to a computerized modeling system; identifying compounds which will bind to the structure; and screening the compounds identified for FabH inhibitory bioactivity.

In a further aspect, the present invention provides an inhibitor of the catalytic activity of any composition bearing the catalytic domain described above.

Another aspect of this invention includes machine readable media encoded with data representing the coordinates of the three-dimensional structure of the FabH crystal.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 provides the atomic coordinates of the *E. coli* FabH dimer.

Fig. 2 provides the atomic coordinates of the *E. coli* FabH monomer in complex with acetyl-CoA.

Fig. 3 provides a projection of the ribbon diagram of the *E. coli* FabH dimer. The two monomers are drawn with a light or dark gray shading. The catalytic Cys112 is shown in dark ball-and-stick model.

Fig. 4 provides the ribbon diagram of the *E. coli* FabH monomer with the catalytic residue Cys112 is shown in dark ball-and-stick model. The N- and C-termini are labeled.

Fig. 5 provides the stereoview of the α-carbon superposition between the structures of FabH and FabF. FabH is drawn in a thin black line and FabF in a thick gray line.

Fig. 6 provides the ribbon diagram of the *E. coli* FabH monomer with acetylated Cys112 and the CoA molecule in black ball-and-stick model. The orientation of the view is the same as that of Fig. 4.

Fig. 7 provides the superposition of the *E. coli* FabH catalytic residues in comparison to those of FabF. FabH is drawn in thick gray lines and FabF in thin black lines. FabH residues are label Cys112, His244 and Asn274, which corresponds to Cys163, His303 and His340, respectively.

Detailed Description of the Invention

The present invention provides a novel *E. coli* FabH crystalline structure, a novel FabH active site, and methods of use of the crystalline form and active site to identify FabH inhibitor compounds (peptide, peptidomimetic or synthetic compositions) characterized by the ability to competitively inhibit binding to the active site of a FabH enzyme. Also provided herein is a novel FabH crystalline structure in complex with the substrate acetyl-CoA, and the identification of acetyl-CoA interacting residues in FabH.

I. The Novel FabH Crystalline Three-Dimensional Structure

The present invention provides a novel FabH crystalline structure based on the *E. coli* FabH. The amino acid sequences of the FabH are provided in Table 1 as SEQ ID NO:1.

TABLE 1

Met Tyr Thr Lys Ile Ile Gly Thr Gly Ser Tyr Leu Pro Glu Gln			
1	5	10	15
Val Arg Thr Asn Ala Asp Leu Glu Lys Met Val Asp Thr Ser Asp			
16	20	25	30
Glu Trp Ile Val Thr Arg Thr Gly Ile Arg Glu Arg His Ile Ala			
31	35	40	45
Ala Pro Asn Glu Thr Val Ser Thr Met Gly Phe Glu Ala Ala Thr			
46	50	55	60
Arg Ala Ile Glu Met Ala Gly Ile Glu Lys Asp Gln Ile Gly Leu			
61	65	70	75
Ile Val Val Ala Thr Thr Ser Ala Thr His Ala Phe Pro Ser Ala			

76 80 85 90
Ala Cys Gln Ile Gln Ser Met Leu Gly Ile Lys Gly Cys Pro Ala
91 95 100 105
Phe Asp Val Ala Ala Ala Cys Ala Gly Phe Thr Tyr Ala Leu Ser
106 110 115 120
Val Ala Asp Gln Tyr Val Lys Ser Gly Ala Val Lys Tyr Ala Leu
121 125 130 135
Val Val Gly Ser Asp Val Leu Ala Arg Thr Cys Asp Pro Thr Asp
136 140 145 150
Arg Gly Thr Ile Ile Ile Phe Gly Asp Gly Ala Gly Ala Ala Val
151 155 160 165
Leu Ala Ala Ser Glu Glu Pro Gly Ile Ile Ser Thr His Leu His
166 170 175 180
Ala Asp Gly Ser Tyr Gly Glu Leu Leu Thr Leu Pro Asn Ala Asp
181 185 190 195
Arg Val Asn Pro Glu Asn Ser Ile His Leu Thr Met Ala Gly Asn
196 200 205 210
Glu Val Phe Lys Val Ala Val Thr Glu Leu Ala His Ile Val Asp
211 215 220 225
Glu Thr Leu Ala Ala Asn Asn Leu Asp Arg Ser Gln Leu Asp Trp
226 230 235 240
Leu Val Pro His Gln Ala Asn Leu Arg Ile Ile Ser Ala Thr Ala
241 245 250 255
Lys Lys Leu Gly Met Ser Met Asp Asn Val Val Val Thr Leu Asp
256 260 265 270
Arg His Gly Asn Thr Ser Ala Ala Ser Val Pro Cys Ala Leu Asp
271 275 280 285
Glu Ala Val Arg Asp Gly Arg Ile Lys Pro Gly Gln Leu Val Leu
286 290 295 300
Leu Glu Ala Phe Gly Gly Gly Phe Thr Trp Gly Ser Ala Leu Val Arg Phe
301 305 310 317

As illustrated herein, the crystal structure is a tightly associated FabH dimer. Each monomer has two structural domains: the N-terminal domain (residues 1-170 of SEQ ID

NO:1) and the C-terminal domain (residues 171-317 of SEQ ID NO:1). The two domains are similar in their overall fold: each contains a 5-stranded β-sheet sandwiched between α-helices and covered by other β-strands, α-helices and loops. The structural similarity between the two halves of the protein indicates that FabH is probably evolved from two genes of similar origin. The active site of FabH is at the center of the FabH monomer, formed at the junction of the N- and C-terminal domains. While the core architecture of the *E. coli* FabH bears some similarity to that of the FabF (Huang, et al, (1998), *EMBO J. 17*, 1183-1191), large differences exit in the atomic positions of the core β-strands, and the structures outside of the core β-strand are completely different. With amino acid sequence identity between FabH and FabF being below 20%, the large differences are well expected. Therefore, the crystalline structure *of E. coli* FabH is novel.

As described above, the *E. coli* FabH is a dimer, each monomer contains an active site. The dimer formation is essential for the FabH activity because the active site of a monomer is comprised of at least Phe87 of the other monomer in the dimer. The present invention provides both a crystalline monomer and dimer structure of *E. coli* FabH. Inhibitors that perturb or interact with this dimer interface are another target for the design and selection of anti-bacterial agents.

According to the present invention, the crystal structure of *E. coli* FabH has been resolved at 2.0 Å (crystal form 1), and its selenomethionine mutant protein in complex with acetyl-CoA has been determined at 1.9 Å (crystal form 2). The structure was determined using the methods of MAD phasing and molecular replacement, and refined to R-factors of 18.9% and 27%, respectively.

Further refinement of the atomic coordinates will change the numbers in Figure 1-2 and Tables I - III, refinement of the crystal structure from another crystal form will result in a new set of coordinates. However, distances and angles in Tables II will remain the same within experimental errors, and relative conformation of residues in the active site will remain the same within experimental error. For example, the two independently determined monomers in our crystal form 1 and the monomer in crystal form 2 do not have identical numerical coordinates, but the structures of these three monomers have very similar structures, and the spatial relationship between amino acid residues are considered the same within experimental error. In fact, we would consider any structure that can be superimposed onto that of FabH with an rms error of less than 1.5 Å on α-carbon atoms being a close structural homologue and the same rms error but over all protein atoms being an identical structure. Figure 1 provides the atomic coordinates of the *E. coli* FabH dimer,

which contains 634 amino acids. Figure 2 provides the atomic coordinates of the *E. coli* FabH monomer in complex with acetyl-CoA, which contains 317 amino acids. The FabH enzyme is characterized by an active site which preferably contains a binding site for the first substrate acetyl-CoA and the second substrate malonyl-ACP. The catalytic residues in FabH are Cys112, His244 and Asn274, compared to Cys163, His303 and His340 in FabF. The difference in catalytic residues is not only limited to their amino acid identity (His340 to Asn274 change), but also their relative spatial arrangement. While FabH Cys112 and Asn274 can be well superimposed onto FabF Cys163 and His340, His244 of FabH occupies a very different position from that of His303 of FabF. This indicated the catalytic mechanisms of the two enzymes are very different. The crystal structure described herein was solved in the presence and absence of acetyl-CoA. We identified that the catalytic Cys112 has been covalently aceytlated, and the product CoA is still bound to the active site. The bound CoA enabled us to identify the active site cavity, which is long and narrow and shaped nicely to bind the β-mercaptoethylamine-patotheinate arm of CoA. The structure of the acetyl-CoA complex also revealed all the key residues that are interacting with CoA and lining the active site, which is identified as a set of 33 amino acid residues listed in Table I. For example, the adenine part of CoA is sandwiched between the side chains of Arg151 and Trp32. Our structures are determined in the absence of malonyl-ACP. However, the same acetyl-CoA binding cavity should bind malonyl-ACP as well because their active site binding regions are very similar and there is no apparent additional entrance to the active site. Moreover, while the FabH molecular surface in general negatively charged, a region just outside of the active site cavity is positively charge. This surface is mainly comprised of three α-helices (30-37, 209-231 and 248-258) and contains a number of positively charged amino acids (Arg36, Arg40, Lys214, His222, Arg235 Arg249, Lys256, Lys257). Since the acyl-carrier protein (ACP) is known to be very acidic or negatively charged, it is reasonable to assume this surface being the ACP binding surface.

Table I provides the the atomic coordinates of the apo *E. coli* FabH structure in the active site (in crystal form 1). Solvent molecules are omitted here for clarity, but can be found in Fig. 1. Residue 487 is Phe87 from the other monomer.

TABLE I

ATOM		RESIDUE		X	Y	Z	Occ	B
1	N	THR	28	-24.151	18.846	61.990	1.00	36.45
2	CA	THR	28	-23.735	19.054	60.610	1.00	36.69
3	CB	THR	28	-22.196	19.086	60.565	1.00	32.66
4	OG1	THR	28	-21.760	20.076	59.636	1.00	33.79
5	CG2	THR	28	-21.645	17.737	60.183	1.00	27.40
6	C	THR	28	-24.238	17.990	59.627	1.00	38.85
7	O	THR	28	-24.732	16.923	60.023	1.00	42.97
8	N	TRP	32	-24.091	20.068	53.681	1.00	30.06
9	CA	TRP	32	-23.725	21.413	54.092	1.00	28.93
10	CB	TRP	32	-24.277	21.708	55.486	1.00	29.27
11	CG	TRP	32	-24.036	23.126	55.939	1.00	31.13
12	CD2	TRP	32	-22.895	23.622	56.644	1.00	32.44
13	CE2	TRP	32	-23.118	25.005	56.890	1.00	35.25
14	CE3	TRP	32	-21.707	23.038	57.096	1.00	32.45
15	CD1	TRP	32	-24.880	24.197	55.779	1.00	33.86
16	NE1	TRP	32	-24.333	25.331	56.351	1.00	35.49
17	CZ2	TRP	32	-22.200	25.800	57.565	1.00	35.24
18	CZ3	TRP	32	-20.793	23.832	57.765	1.00	34.43
19	CH2	TRP	32	-21.046	25.197	57.994	1.00	36.72
20	C	TRP	32	-22.203	21.582	54.091	1.00	27.24
21	O	TRP	32	-21.675	22.617	53.674	1.00	26.75
22	N	ILE	33	-21.503	20.566	54.581	1.00	26.32
23	CA	ILE	33	-20.042	20.617	54.642	1.00	25.89
24	CB	ILE	33	-19.459	19.370	55.333	1.00	25.18
25	CG2	ILE	33	-17.925	19.444	55.366	1.00	26.64
26	CG1	ILE	33	-20.024	19.253	56.744	1.00	18.01
27	CD1	ILE	33	-19.621	18.008	57.421	1.00	19.10
28	C	ILE	33	-19.432	20.755	53.258	1.00	24.76
29	O	ILE	33	-18.630	21.650	53.022	1.00	23.20
30	N	ARG	36	-20.198	24.159	51.621	1.00	26.35
31	CA	ARG	36	-19.545	25.296	52.237	1.00	27.73
32	CB	ARG	36	-20.083	25.473	53.649	1.00	34.96
33	CG	ARG	36	-19.562	26.715	54.326	1.00	47.48
34	CD	ARG	36	-20.581	27.250	55.290	1.00	56.04
35	NE	ARG	36	-21.775	27.729	54.600	1.00	63.48
36	CZ	ARG	36	-22.490	28.780	54.996	1.00	67.12
37	NH1	ARG	36	-23.564	29.153	54.303	1.00	67.75
38	NH2	ARG	36	-22.127	29.465	56.082	1.00	68.27

TABLE I - Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
39	C	ARG	36	-18.014	25.292	52.233	1.00	23.26
40	O	ARG	36	-17.386	26.346	52.208	1.00	21.26
41	N	THR	37	-17.423	24.103	52.214	1.00	20.79
42	CA	THR	37	-15.973	23.969	52.258	1.00	19.72
43	CB	THR	37	-15.549	23.164	53.509	1.00	20.01
44	OG1	THR	37	-16.014	21.812	53.384	1.00	17.59
45	CG2	THR	37	-16.157	23.752	54.765	1.00	18.21
46	C	THR	37	-15.363	23.272	51.047	1.00	20.77
47	O	THR	37	-14.234	23.571	50.657	1.00	20.98
48	N	CYS	112	-0.698	28.695	58.467	1.00	12.58
49	CA	CYS	112	-0.984	28.096	57.174	1.00	11.86
50	CB	CYS	112	-2.457	28.264	56.808	1.00	10.86
51	SG	CYS	112	-3.580	27.460	57.935	1.00	22.06
52	C	CYS	112	-0.126	28.620	56.037	1.00	10.86
53	O	CYS	112	-0.003	27.939	55.025	1.00	13.89
54	N	LEU	142	-3.033	20.066	62.705	1.00	16.58
55	CA	LEU	142	-4.063	20.954	63.207	1.00	17.95
56	CB	LEU	142	-4.281	22.159	62.287	1.00	15.72
57	CG	LEU	142	-3.100	23.125	62.126	1.00	18.13
58	CD1	LEU	142	-3.628	24.499	61.738	1.00	14.84
59	CD2	LEU	142	-2.246	23.204	63.415	1.00	12.26
60	C	LEU	142	-5.396	20.321	63.598	1.00	17.45
61	O	LEU	142	-6.111	20.883	64.417	1.00	17.68
62	N	ARG	151	-17.927	23.092	65.249	1.00	22.20
63	CA	ARG	151	-18.230	22.887	63.841	1.00	25.49
64	CB	ARG	151	-19.699	23.217	63.534	1.00	24.14
65	CG	ARG	151	-20.051	22.998	62.052	1.00	33.87
66	CD	ARG	151	-21.530	23.158	61.748	1.00	37.44
67	NE	ARG	151	-21.991	24.545	61.780	1.00	41.79
68	CZ	ARG	151	-23.272	24.897	61.737	1.00	44.63
69	NH1	ARG	151	-23.612	26.173	61.771	1.00	46.51
70	NH2	ARG	151	-24.219	23.970	61.666	1.00	47.88
71	C	ARG	151	-17.304	23.634	62.868	1.00	26.00
72	O	ARG	151	-16.686	23.018	61.992	1.00	26.64
73	N	GLY	152	-17.164	24.940	63.077	1.00	24.63
74	CA	GLY	152	-16.353	25.769	62.201	1.00	23.08
75	C	GLY	152	-14.912	25.371	61.944	1.00	22.21
76	O	GLY	152	-14.366	25.679	60.880	1.00	21.32
77	N	ILE	155	-14.484	20.649	60.878	1.00	18.82
78	CA	ILE	155	-14.866	20.149	59.564	1.00	18.77
79	CB	ILE	155	-16.223	20.733	59.071	1.00	17.77
80	CG2	ILE	155	-17.365	20.321	60.018	1.00	12.79

TABLE I - Cont.

	ATOM		RESIDUE		X	Y	Z	Occ	B
	81	CG1	ILE	155	-16.127	22.249	58.924	1.00	15.46
	82	CD1	ILE	155	-17.339	22.892	58.331	1.00	20.95
5	83	C	ILE	155	-13.823	20.489	58.531	1.00	18.45
	84	O	ILE	155	-13.819	19.909	57.461	1.00	21.51
	85	N	ILE	156	-12.958	21.450	58.819	1.00	18.70
	86	CA	ILE	156	-11.985	21.825	57.812	1.00	19.10
	87	CB	ILE	156	-11.999	23.375	57.499	1.00	24.79
10	88	CG2	ILE	156	-13.391	23.974	57.563	1.00	23.59
	89	CG1	ILE	156	-11.095	24.139	58.438	1.00	24.77
	90	CD1	ILE	156	-9.886	24.631	57.730	1.00	27.97
	91	C	ILE	156	-10.544	21.338	57.935	1.00	18.32
	92	O	ILE	156	-9.922	21.071	56.918	1.00	18.31
15	93	N	PHE	157	-10.005	21.200	59.142	1.00	16.26
	94	CA	PHE	157	-8.611	20.780	59.280	1.00	15.33
	95	CB	PHE	157	-7.984	21.371	60.551	1.00	15.71
	96	CG	PHE	157	-7.868	22.858	60.523	1.00	19.05
	97	CD1	PHE	157	-8.814	23.654	61.158	1.00	19.74
20	98	CD2	PHE	157	-6.844	23.476	59.814	1.00	15.77
	99	CE1	PHE	157	-8.737	25.057	61.076	1.00	21.28
	100	CE2	PHE	157	-6.761	24.855	59.727	1.00	11.63
	101	CZ	PHE	157	-7.701	25.650	60.351	1.00	17.65
	102	C	PHE	157	-8.278	19.286	59.190	1.00	16.07
25	103	O	PHE	157	-9.045	18.413	59.622	1.00	17.36
	104	N	LEU	189	-7.786	34.391	64.172	1.00	19.01
	105	CA	LEU	189	-7.338	33.021	63.922	1.00	19.46
	106	CB	LEU	189	-6.897	32.907	62.463	1.00	23.06
	107	CG	LEU	189	-6.422	31.587	61.872	1.00	23.21
30	108	CD1	LEU	189	-7.435	30.493	62.157	1.00	24.24
	109	CD2	LEU	189	-6.253	31.811	60.355	1.00	25.52
	110	C	LEU	189	-6.164	32.746	64.850	1.00	18.26
	111	O	LEU	189	-5.082	33.338	64.688	1.00	15.62
	112	N	LEU	205	-7.765	25.549	68.834	1.00	19.58
35	113	CA	LEU	205	-7.699	26.448	67.677	1.00	19.69
	114	CB	LEU	205	-7.475	25.601	66.398	1.00	19.66
	115	CG	LEU	205	-7.104	26.238	65.052	1.00	19.36
	116	CD1	LEU	205	-6.309	25.259	64.201	1.00	18.01
	117	CD2	LEU	205	-8.366	26.671	64.321	1.00	18.66
40	118	C	LEU	205	-8.996	27.273	67.597	1.00	17.98
	119	O	LEU	205	-10.088	26.731	67.804	1.00	20.78
	120	N	MET	207	-11.189	30.405	65.330	1.00	16.50
	121	CA	MET	207	-11.285	31.040	64.025	1.00	18.56
	122	CB	MET	207	-11.105	30.003	62.931	1.00	20.76

TABLE I - Cont.

	ATOM		RESIDUE		X	Y	Z	Occ	B
	123	CG	MET	207	-11.293	30.550	61.542	1.00	23.66
	124	SD	MET	207	-10.858	29.292	60.353	1.00	32.43
5	125	CE	MET	207	-12.262	28.166	60.555	1.00	31.26
	126	C	MET	207	-12.599	31.742	63.776	1.00	18.83
	127	O	MET	207	-13.666	31.152	63.934	1.00	19.82
	128	N	GLY	209	-14.190	32.425	61.134	1.00	20.42
	129	CA	GLY	209	-14.305	32.056	59.737	1.00	23.52
10	130	C	GLY	209	-14.623	33.114	58.701	1.00	24.44
	131	O	GLY	209	-13.771	33.456	57.884	1.00	26.52
	132	N	ASN	210	-15.839	33.640	58.738	1.00	23.37
	133	CA	ASN	210	-16.291	34.615	57.758	1.00	25.94
	134	CB	ASN	210	-17.724	35.006	58.035	1.00	24.49
15	135	CG	ASN	210	-18.633	33.818	58.029	1.00	25.13
	136	OD1	ASN	210	-18.680	33.068	57.061	1.00	25.86
	137	ND2	ASN	210	-19.325	33.603	59.130	1.00	25.81
	138	C	ASN	210	-15.426	35.831	57.639	1.00	26.62
	139	O	ASN	210	-15.214	36.334	56.545	1.00	27.59
20	140	N	VAL	212	-12.110	35.950	58.414	1.00	25.15
	141	CA	VAL	212	-10.808	35.645	57.793	1.00	25.13
	142	CB	VAL	212	-10.004	34.469	58.486	1.00	23.52
	143	CG1	VAL	212	-10.492	34.190	59.896	1.00	20.23
	144	CG2	VAL	212	-9.958	33.220	57.653	1.00	23.20
25	145	C	VAL	212	-10.971	35.405	56.272	1.00	23.49
	146	O	VAL	212	-10.095	35.769	55.493	1.00	20.62
	147	N	PHE	213	-12.115	34.859	55.853	1.00	22.05
	148	CA	PHE	213	-12.371	34.627	54.431	1.00	22.19
	149	CB	PHE	213	-13.718	33.954	54.244	1.00	20.95
30	150	CG	PHE	213	-14.116	33.771	52.794	1.00	23.47
	151	CD1	PHE	213	-14.758	34.788	52.101	1.00	22.38
	152	CD2	PHE	213	-13.833	32.587	52.132	1.00	21.51
	153	CE1	PHE	213	-15.098	34.634	50.784	1.00	23.71
	154	CE2	PHE	213	-14.173	32.423	50.813	1.00	26.06
35	155	CZ	PHE	213	-14.805	33.446	50.133	1.00	25.34
	156	C	PHE	213	-12.307	35.935	53.645	1.00	22.07
	157	O	PHE	213	-11.618	36.045	52.629	1.00	22.83
	158	N	ALA	216	-8.801	37.118	53.586	1.00	18.14
	159	CA	ALA	216	-7.964	36.216	52.808	1.00	19.00
40	160	CB	ALA	216	-8.183	34.775	53.218	1.00	17.94
	161	C	ALA	216	-8.146	36.371	51.303	1.00	17.97
	162	O	ALA	216	-7.166	36.285	50.563	1.00	16.52
	163	N	LEU	220	-4.879	37.537	49.135	1.00	15.55
	164	CA	LEU	220	-4.379	36.571	48.174	1.00	17.76

TABLE I - Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
165	CB	LEU	220	-5.127	35.233	48.275	1.00	15.75
166	CG	LEU	220	-4.703	34.362	49.466	1.00	13.65
167	CD1	LEU	220	-5.621	33.177	49.608	1.00	13.89
168	CD2	LEU	220	-3.278	33.915	49.310	1.00	9.45
169	C	LEU	220	-4.491	37.186	46.769	1.00	17.28
170	O	LEU	220	-3.618	36.957	45.932	1.00	20.62
171	N	HIS	244	-3.012	27.197	48.689	1.00	18.90
172	CA	HIS	244	-3.165	26.587	49.988	1.00	17.18
173	CB	HIS	244	-2.914	27.594	51.111	1.00	17.15
174	CG	HIS	244	-3.178	27.035	52.465	1.00	17.42
175	CD2	HIS	244	-2.579	26.015	53.138	1.00	14.15
176	ND1	HIS	244	-4.285	27.385	53.212	1.00	16.36
177	CE1	HIS	244	-4.370	26.596	54.264	1.00	16.12
178	NE2	HIS	244	-3.354	25.760	54.244	1.00	19.14
179	C	HIS	244	-4.631	26.151	49.971	1.00	15.41
180	O	HIS	244	-5.503	26.936	49.591	1.00	14.10
181	N	ALA	246	-7.440	26.051	51.721	1.00	19.76
182	CA	ALA	246	-8.240	26.512	52.864	1.00	20.02
183	CB	ALA	246	-8.166	28.017	52.975	1.00	22.28
184	C	ALA	246	-9.687	26.075	52.772	1.00	23.08
185	O	ALA	246	-10.281	25.620	53.759	1.00	23.37
186	N	ASN	247	-10.280	26.349	51.615	1.00	21.20
187	CA	ASN	247	-11.645	25.983	51.311	1.00	22.48
188	CB	ASN	247	-12.653	26.733	52.190	1.00	24.84
189	CG	ASN	247	-12.700	28.195	51.888	1.00	26.54
190	OD1	ASN	247	-13.343	28.618	50.942	1.00	32.63
191	ND2	ASN	247	-12.016	28.987	52.686	1.00	31.62
192	C	ASN	247	-11.824	26.292	49.825	1.00	23.43
193	O	ASN	247	-11.076	27.097	49.249	1.00	23.61
194	N	ARG	249	-14.126	27.939	48.119	1.00	27.79
195	CA	ARG	249	-14.566	29.305	47.811	1.00	28.98
196	CB	ARG	249	-15.376	29.912	48.966	1.00	34.43
197	CG	ARG	249	-16.577	29.118	49.433	1.00	45.16
198	CD	ARG	249	-17.307	29.859	50.557	1.00	52.72
199	NE	ARG	249	-18.235	30.862	50.037	1.00	60.09
200	CZ	ARG	249	-18.607	31.976	50.675	1.00	62.73
201	NH1	ARG	249	-19.469	32.803	50.096	1.00	64.82
202	NH2	ARG	249	-18.112	32.290	51.867	1.00	60.24
203	C	ARG	249	-13.369	30.208	47.562	1.00	24.80
204	O	ARG	249	-13.358	31.007	46.629	1.00	24.09
205	N	ILE	250	-12.393	30.135	48.453	1.00	24.48
206	CA	ILE	250	-11.201	30.951	48.306	1.00	24.93

TABLE I - Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
207	CB	ILE	250	-10.365	30.965	49.621	1.00	26.91
208	CG2	ILE	250	-8.880	31.128	49.350	1.00	22.69
209	CG1	ILE	250	-10.902	32.091	50.506	1.00	32.57
210	CD1	ILE	250	-10.216	32.245	51.828	1.00	38.42
211	C	ILE	250	-10.391	30.533	47.076	1.00	23.12
212	O	ILE	250	-10.024	31.380	46.265	1.00	20.24
213	N	ASN	274	-7.884	20.993	55.104	1.00	16.04
214	CA	ASN	274	-6.887	22.042	55.213	1.00	16.17
215	CB	ASN	274	-7.524	23.307	55.790	1.00	16.71
216	CG	ASN	274	-6.524	24.433	56.031	1.00	15.26
217	OD1	ASN	274	-5.290	24.259	55.970	1.00	14.69
218	ND2	ASN	274	-7.058	25.607	56.319	1.00	17.12
219	C	ASN	274	-5.800	21.538	56.144	1.00	18.93
220	O	ASN	274	-6.016	21.456	57.366	1.00	18.02
221	N	SER	276	-2.883	23.010	56.745	1.00	14.34
222	CA	SER	276	-1.996	24.086	57.152	1.00	14.51
223	CB	SER	276	-1.772	23.993	58.686	1.00	16.80
224	OG	SER	276	-1.051	25.104	59.218	1.00	17.07
225	C	SER	276	-0.675	24.141	56.352	1.00	13.90
226	O	SER	276	-0.719	24.199	55.132	1.00	15.64
227	N	ALA	303	-0.360	31.072	49.683	1.00	15.48
228	CA	ALA	303	-0.934	30.617	50.937	1.00	13.31
229	CB	ALA	303	0.045	29.692	51.624	1.00	11.10
230	C	ALA	303	-1.261	31.801	51.853	1.00	12.59
231	O	ALA	303	-0.614	32.842	51.789	1.00	11.22
232	N	PHE	304	-2.299	31.642	52.666	1.00	14.82
233	CA	PHE	304	-2.726	32.650	53.626	1.00	15.34
234	CB	PHE	304	-4.075	33.248	53.207	1.00	17.57
235	CG	PHE	304	-4.561	34.355	54.119	1.00	23.99
236	CD1	PHE	304	-5.356	34.060	55.243	1.00	22.77
237	CD2	PHE	304	-4.220	35.687	53.866	1.00	22.34
238	CE1	PHE	304	-5.794	35.064	56.089	1.00	19.22
239	CE2	PHE	304	-4.657	36.705	54.712	1.00	24.60
240	CZ	PHE	304	-5.447	36.389	55.826	1.00	19.92
241	C	PHE	304	-2.831	31.946	54.982	1.00	15.89
242	O	PHE	304	-3.176	30.768	55.041	1.00	14.69
243	N	GLY	305	-2.490	32.637	56.065	1.00	14.20
244	CA	GLY	305	-2.583	32.002	57.363	1.00	13.74
245	C	GLY	305	-2.765	32.889	58.578	1.00	13.32
246	O	GLY	305	-2.788	34.115	58.496	1.00	12.57
247	N	GLY	306	-2.856	32.235	59.727	1.00	17.80

TABLE I - Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
248	CA	GLY	306	-3.033	32.929	60.990	1.00	17.87
249	C	GLY	306	-1.928	33.892	61.357	1.00	19.45
250	O	GLY	306	-0.758	33.751	60.965	1.00	19.00
251	N	PHE	487	0.118	30.521	66.721	1.00	16.05
252	CA	PHE	487	-0.254	31.597	65.800	1.00	15.49
253	CB	PHE	487	-0.539	31.168	64.330	1.00	10.60
254	CG	PHE	487	-1.559	30.100	64.167	1.00	9.77
255	CD1	PHE	487	-1.169	28.788	63.944	1.00	11.44
256	CD2	PHE	487	-2.916	30.410	64.157	1.00	12.27
257	CE1	PHE	487	-2.109	27.796	63.715	1.00	10.46
258	CE2	PHE	487	-3.878	29.416	63.925	1.00	11.90
259	CZ	PHE	487	-3.477	28.111	63.705	1.00	9.96
260	C	PHE	487	-1.381	32.376	66.460	1.00	13.45
261	O	PHE	487	-2.233	31.776	67.132	1.00	15.58

Table II provides the distances between (D) atoms of the active site residues that are within 5.0 angstroms of one another as defined by Table I.

TABLE II

Atom 1	Atom 2	Distance Between (D=)	Atom 1	Atom 2	Distance Between (D=)
28CA	151CD	4.796	32N	33CA	4.198
28CB	33CD1	4.204	32N	33C	4.728
28CB	151CD	4.292	32N	33CB	4.967
28CB	33CG1	4.398	32CA	33N	2.428
28CB	151CG	4.703	32CA	33CA	3.808
28OG1	33CG1	3.472	32CA	33C	4.422
28OG1	33CD1	3.709	32CA	33CB	4.890
28OG1	151CD	3.743	32CB	33N	3.133
28OG1	32CE3	3.902	32CB	33CA	4.454
28OG1	151CG	4.159	32CG	33N	3.849
28OG1	32CZ3	4.306	32CG	33CA	4.892
28OG1	155CG2	4.418	32CD2	33N	3.941
28OG1	32CD2	4.776	32CD2	36CB	4.506
28OG1	33CB	4.930	32CD2	36CD	4.511
28OG1	151NE	4.962	32CD2	33CA	4.602
28CG2	33CD1	3.435	32CD2	36NE	4.722
28CG2	33CG1	4.093	32CE2	36CD	3.747
32N	33N	2.785	32CE2	36NE	3.804

	32CE2	36CZ	4.270
	32CE2	36CB	4.465
	32CE2	36NH2	4.640
	32CE2	36CG	4.706
5	32CE2	151CZ	4.851
	32CE2	36NH1	4.909
	32CE3	33N	3.532
	32CE3	33CA	3.828
	32CE3	33CG1	4.157
10	32CE3	36CB	4.522
	32CE3	155CD1	4.542
	32CE3	33CB	4.649
	32CE3	151CD	4.657
	32CE3	36CD	4.719
15	32CE3	151NE	4.929
	32CD1	36NE	4.848
	32NE1	36NE	3.919
	32NE1	36CZ	4.139
	32NE1	36CD	4.346
20	32NE1	36NH1	4.404
	32NE1	36NH2	4.693
	32CZ2	36CD	3.146
	32CZ2	36NE	3.563
	32CZ2	36CZ	3.945
25	32CZ2	36NH2	3.954
	32CZ2	36CG	4.276
	32CZ2	151CZ	4.401
	32CZ2	151NE	4.403
	32CZ2	151NH1	4.452
30	32CZ2	36CB	4.464
	32CZ2	36NH1	4.873
	32CZ2	151NH2	4.924
	32CZ2	151CD	4.993
	32CZ3	155CD1	3.624
35	32CZ3	151CD	4.106
	32CZ3	36CD	4.225
	32CZ3	151NE	4.250
	32CZ3	151CG	4.430
	32CZ3	36CB	4.488
40	32CZ3	33CA	4.545
	32CZ3	33N	4.616
	32CZ3	36CG	4.653
	32CZ3	33CG1	4.754
	32CZ3	151CZ	4.802
45	32CH2	36CD	3.427
	32CH2	151NE	3.956
	32CH2	36CG	4.238
	32CH2	36NE	4.297
	32CH2	151CD	4.299
50	32CH2	151CZ	4.365
	32CH2	155CD1	4.378
	32CH2	36CB	4.459
	32CH2	151NH1	4.669
	32CH2	151CG	4.722
55	32CH2	36NH2	4.800
	32CH2	36CZ	4.890
	32C	33CA	2.430
	32C	33C	3.009
	32C	33O	3.730
60	32C	33CB	3.737
	32C	36N	4.094
	32C	33CG1	4.149
	32C	36CB	4.453
	32C	36CA	4.929
65	32C	33CG2	4.950
	32O	33N	2.249
	32O	33CA	2.757
	32O	33C	2.945
	32O	36N	2.962
70	32O	33O	3.261
	32O	36CB	3.270
	32O	36CA	3.712
	32O	33CB	4.267
	32O	36CG	4.657
75	32O	37N	4.735
	32O	36C	4.758
	32O	33CG1	4.844
	33N	36N	4.835
	33CA	37OG1	4.386
80	33CA	36N	4.658
	33CA	36CB	4.957
	33CA	37N	4.991
	33CA	37CG2	4.994
	33CB	37OG1	4.651

	33CG2	37OG1	3.631
	33CG2	155CB	4.276
	33CG2	155CD1	4.585
	33CG2	155O	4.633
5	33CG2	37CG2	4.695
	33CG2	155CG2	4.767
	33CG2	37CB	4.789
	33CG2	155CG1	4.874
	33CG1	155CG2	4.351
10	33CG1	155CB	4.696
	33CG1	155CD1	4.793
	33CD1	155CG2	4.145
	33CD1	155CB	4.658
	33C	37OG1	3.580
15	33C	36N	3.854
	33C	37N	4.042
	33C	37CB	4.576
	33C	36CA	4.656
	33C	37CG2	4.688
20	33C	36CB	4.779
	33C	37CA	4.826
	33C	36C	4.863
	33O	37OG1	2.646
	33O	37N	2.851
25	33O	36N	3.274
	33O	37CB	3.467
	33O	37CA	3.608
	33O	37CG2	3.684
	33O	36C	3.777
30	33O	36CA	3.840
	33O	36CB	4.138
	33O	37C	4.148
	33O	36O	4.926
	36N	37N	2.838
35	36N	37CA	4.277
	36N	37C	4.949
	36CA	37N	2.434
	36CA	37CA	3.811
	36CA	37CG2	4.500
40	36CA	37CB	4.704
	36CA	37C	4.796
	36CB	37N	3.318
	36CB	37CG2	4.430
	36CB	37CA	4.592
45	36CG	37N	3.982
	36CG	37CG2	4.535
	36CG	37CA	4.970
	36C	37CA	2.432
	36C	37CG2	3.497
50	36C	37CB	3.498
	36C	37C	3.538
	36C	37OG1	4.176
	36C	37O	4.442
	36C	249CD	4.916
55	36C	249CG	4.954
	36O	37N	2.243
	36O	37CA	2.766
	36O	37CG2	3.844
	36O	37C	3.859
60	36O	249CD	3.882
	36O	37CB	3.898
	36O	249CG	4.005
	36O	37O	4.477
	36O	247CB	4.749
65	36O	247OD1	4.807
	36O	37OG1	4.881
	37CA	247CB	4.321
	37CA	247CA	4.867
	37CB	156CG2	4.663
70	37CB	247CB	4.782
	37CG2	155CD1	3.854
	37CG2	156CG2	3.941
	37CG2	155CG1	4.422
	37CG2	156CB	4.991
75	37C	247CB	4.542
	37C	247CA	4.609
	37C	247C	4.810
	37O	247CA	3.598
	37O	247C	3.729
80	37O	247CB	3.853
	37O	247N	4.926
	37O	247O	4.938
	112N	276OG	3.686
	112N	305CA	3.963

	112N	306N	4.333
	112N	305C	4.677
	112N	304O	4.709
	112N	276CB	4.828
5	112N	305N	4.952
	112N	276CA	4.966
	112CA	276OG	3.624
	112CA	276C	4.051
	112CA	304O	4.061
10	112CA	276CA	4.136
	112CA	305CA	4.225
	112CA	276O	4.408
	112CA	244NE2	4.434
	112CA	276CB	4.443
15	112CA	244CE1	4.710
	112CA	304C	4.800
	112CA	244CD2	4.813
	112CA	305N	4.911
	112CB	304O	3.148
20	112CB	244CE1	3.594
	112CB	244NE2	3.694
	112CB	305CA	3.781
	112CB	304C	4.127
	112CB	244ND1	4.129
25	112CB	276OG	4.216
	112CB	276CA	4.217
	112CB	244CD2	4.306
	112CB	305N	4.436
	112CB	276C	4.515
30	112CB	244CG	4.571
	112CB	276CB	4.716
	112CB	276O	4.728
	112CB	306N	4.945
	112CB	305C	4.962
35	112CB	274OD1	4.977
	112SG	276OG	3.687
	112SG	276CA	3.809
	112SG	244CE1	3.853
	112SG	276CB	3.982
40	112SG	244NE2	4.070
	112SG	274OD1	4.127
	112SG	274ND2	4.259
	112SG	304O	4.414
	112SG	157CE2	4.485
45	112SG	274CG	4.632
	112SG	276N	4.659
	112SG	305CA	4.685
	112SG	276C	4.686
	112SG	244ND1	4.776
50	112SG	142CD1	4.820
	112C	304O	3.861
	112C	305CA	4.386
	112C	304C	4.415
	112C	276C	4.524
55	112C	303CB	4.545
	112C	276O	4.551
	112C	244CD2	4.605
	112C	305N	4.661
	112C	244NE2	4.671
60	112C	276OG	4.831
	112C	244CG	4.958
	112O	244CD2	3.728
	112O	276O	3.809
	112O	303CB	3.827
65	112O	244NE2	4.073
	112O	276C	4.079
	112O	244CG	4.177
	112O	304O	4.251
	112O	244CE1	4.632
70	112O	244ND1	4.683
	112O	276CA	4.831
	112O	244CB	4.890
	112O	304C	4.905
	112O	304N	4.955
75	112O	303CA	4.975
	142CA	157CB	4.754
	142CA	205CD1	4.956
	142CB	157CD2	3.797
	142CB	157CG	4.058
80	142CB	157CB	4.165
	142CB	205CD1	4.170
	142CB	157CE2	4.469
	142CB	276CB	4.757
	142CB	157CD1	4.905

	142CG	276CB	3.788
	142CG	276OG	4.071
	142CG	205CD1	4.377
	142CG	157CD2	4.414
5	142CG	157CE2	4.706
	142CD1	276CB	3.608
	142CD1	276OG	3.655
	142CD1	205CD1	3.719
	142CD1	157CE2	3.740
10	142CD1	157CD2	3.885
	142CD1	487CZ	4.116
	142CD1	487CE1	4.134
	142CD1	157CZ	4.454
	142CD1	157CG	4.706
15	142CD1	276CA	4.885
	142CD2	487CE1	4.604
	142CD2	205CD1	4.620
	142CD2	276OG	4.759
	142CD2	276CB	4.818
20	142C	157CB	4.133
	142C	157CG	4.691
	142O	157CB	4.323
	142O	205CD1	4.386
	142O	157CG	4.706
25	151N	152N	2.952
	151N	152CA	4.351
	151CA	152N	2.436
	151CA	152CA	3.810
	151CA	152C	4.558
30	151CA	155CG2	4.685
	151CB	152N	3.099
	151CB	152CA	4.414
	151CG	152N	3.627
	151CG	155CG2	4.303
35	151CG	155CD1	4.606
	151CG	152CA	4.623
	151CD	152N	4.899
	151C	152CA	2.431
	151C	152C	3.097
40	151C	152O	4.095
	151C	155CG1	4.343
	151C	155CG2	4.371
	151C	155N	4.563
	151C	155CD1	4.597
45	151C	155CB	4.899
	151O	152N	2.258
	151O	152CA	2.779
	151O	152C	2.947
	151O	155CG1	3.212
50	151O	155CG2	3.411
	151O	155N	3.421
	151O	152O	3.701
	151O	155CD1	3.721
	151O	155CB	3.737
55	151O	155CA	4.176
	152CA	155CG1	4.815
	152CA	155CD1	4.922
	152C	207CE	4.094
	152C	155CG1	4.510
60	152C	156CG2	4.843
	152C	155N	4.860
	152O	207CE	3.274
	152O	156CG2	3.855
	152O	155CG1	4.323
65	152O	156CG1	4.363
	152O	156CB	4.727
	152O	155CD1	4.807
	152O	156N	4.911
	155N	156N	2.685
70	155N	156CA	4.127
	155N	156CG2	4.821
	155N	157N	4.835
	155N	156C	4.966
	155CA	156N	2.427
75	155CA	156CA	3.765
	155CA	156CG2	4.562
	155CA	156C	4.769
	155CA	156CB	4.784
	155CA	157N	4.991
80	155CB	156N	3.352
	155CB	156CA	4.554
	155CB	156CG2	4.561
	155CG2	156N	4.705
	155CG1	156N	3.270

	155CG1	156CG2	3.509
	155CG1	156CA	4.310
	155CG1	156CB	4.510
	155CD1	156CG2	4.165
5	155CD1	156N	4.638
	155C	156CA	2.383
	155C	156C	3.439
	155C	156CB	3.567
	155C	156CG2	3.643
10	155C	157N	3.931
	155C	156O	4.261
	155C	156CG1	4.558
	155O	156N	2.227
	155O	156CA	2.675
15	155O	156C	3.604
	155O	156CB	3.915
	155O	156CG2	4.089
	155O	156O	4.103
	155O	157N	4.363
20	156N	157N	2.981
	156N	157CA	4.422
	156CA	157N	2.466
	156CA	157CA	3.825
	156CA	157C	4.700
25	156CA	157O	4.854
	156CA	157CB	4.870
	156CA	157CD1	4.959
	156CA	274N	4.980
	156CB	157N	3.377
30	156CB	157CA	4.624
	156CB	246O	4.688
	156CB	274CB	4.791
	156CB	157CD1	4.859
	156CG2	157N	4.653
35	156CG1	157N	3.213
	156CG1	157CD1	3.583
	156CG1	157CE1	3.655
	156CG1	157CG	4.050
	156CG1	157CZ	4.179
40	156CG1	157CA	4.262
	156CG1	157CD2	4.517
	156CG1	274CB	4.523
	156CG1	157CE2	4.578
	156CG1	157CB	4.670
45	156CG1	207CE	4.697
	156CG1	274ND2	4.790
	156CG1	246O	4.975
	156CD1	274ND2	3.308
	156CD1	274CB	3.331
50	156CD1	157CZ	3.561
	156CD1	157CE1	3.563
	156CD1	157N	3.712
	156CD1	157CE2	3.715
	156CD1	157CD1	3.722
55	156CD1	274CG	3.772
	156CD1	157CD2	3.864
	156CD1	157CG	3.875
	156CD1	246O	4.111
	156CD1	157CA	4.343
60	156CD1	274CA	4.694
	156CD1	157CB	4.712
	156CD1	274N	4.913
	156CD1	274OD1	4.936
	156C	157N	1.329
65	156C	157CA	2.420
	156C	157C	3.305
	156C	157CB	3.660
	156C	157O	3.694
	156C	274N	3.900
70	156C	157CG	4.021
	156C	274CB	4.195
	156C	157CD1	4.329
	156C	274O	4.565
	156C	274CA	4.613
75	156C	157CD2	4.668
	156O	157N	2.229
	156O	157CA	2.717
	156O	274N	2.729
	156O	157C	3.324
80	156O	274CB	3.467
	156O	274CA	3.614
	156O	157O	3.892
	156O	274O	3.950
	156O	157CB	4.129

	156O	274C	4.220
	156O	157CG	4.518
	156O	274CG	4.862
	156O	157CD2	4.863
5	157N	274O	4.374
	157N	274N	4.566
	157N	274CB	4.672
	157CA	274O	3.295
	157CA	274N	4.244
10	157CA	274C	4.279
	157CA	274CB	4.444
	157CA	274CA	4.594
	157CB	274O	3.745
	157CB	274C	4.921
15	157CG	274O	3.919
	157CG	205CD1	4.661
	157CG	274CB	4.767
	157CG	274CG	4.946
	157CD1	205CD1	4.256
20	157CD1	205CD2	4.394
	157CD1	205CG	4.976
	157CD2	274O	3.280
	157CD2	274CG	3.915
	157CD2	274CB	4.085
25	157CD2	274ND2	4.099
	157CD2	274OD1	4.220
	157CD2	274C	4.280
	157CD2	205CD1	4.766
	157CD2	274CA	4.819
30	157CE1	205CD2	3.643
	157CE1	205CD1	3.963
	157CE1	205CG	4.458
	157CE1	207CE	4.729
	157CE1	207SD	4.791
35	157CE2	274ND2	3.503
	157CE2	274CG	3.728
	157CE2	274OD1	4.078
	157CE2	274O	4.205
	157CE2	274CB	4.299
40	157CE2	205CD1	4.515
	157CE2	274C	4.976
	157CZ	274ND2	4.083
	157CZ	205CD1	4.113
	157CZ	205CD2	4.153
45	157CZ	274CG	4.640
	157CZ	205CG	4.775
	157CZ	207SD	4.820
	157C	274O	3.627
	157C	274N	4.446
50	157C	274C	4.527
	157O	274O	4.850
	189N	207CA	4.847
	189CA	207CA	4.417
	189CA	207N	4.864
55	189CA	207CB	4.928
	189CA	487CE2	4.997
	189CB	306CA	4.135
	189CB	212CG1	4.600
	189CB	487CE2	4.841
60	189CB	306N	4.926
	189CB	487CD2	4.995
	189CG	306CA	3.750
	189CG	487CE2	3.924
	189CG	306N	4.212
65	189CG	487CD2	4.347
	189CG	487CZ	4.911
	189CG	207CG	4.991
	189CD1	207CB	3.783
	189CD1	207CG	3.907
70	189CD1	207SD	4.051
	189CD1	487CE2	4.116
	189CD1	207CA	4.314
	189CD1	205CD2	4.490
	189CD1	487CZ	4.872
75	189CD1	207N	4.916
	189CD1	487CD2	4.942
	189CD2	306CA	3.467
	189CD2	306N	3.480
	189CD2	305C	4.060
80	189CD2	305O	4.557
	189CD2	305CA	4.739
	189CD2	212CG2	4.797
	189CD2	212CG1	4.883
	189CD2	306C	4.903

	189CD2	487CE2	4.911
	189CD2	212CB	4.963
	189C	487CD2	4.060
	189C	487CE2	4.144
5	189C	487O	4.648
	189C	306CA	4.974
	189O	487CD2	3.681
	189O	487O	4.066
	189O	487CE2	4.173
10	189O	487C	4.215
	189O	306CA	4.247
	189O	306C	4.621
	189O	487CG	4.813
	205CG	487CZ	4.299
15	205CG	487CE2	4.667
	205CD1	487CZ	4.050
	205CD1	487CE2	4.824
	205CD1	487CE1	4.931
	205CD2	207CB	4.532
20	205CD2	207N	4.789
	205C	207N	4.445
	205O	207N	4.564
	207CA	209N	4.326
	207CB	209N	4.314
25	207CB	209CA	4.966
	207CG	209N	3.475
	207CG	209CA	3.821
	207CG	212CG1	4.074
	207CG	212CG2	4.903
30	207SD	209CA	4.461
	207SD	209N	4.640
	207SD	212CG2	4.851
	207SD	212CG1	4.933
	207CE	209CA	4.469
35	207CE	209N	4.711
	207C	209N	3.159
	207C	209CA	4.396
	207O	209N	3.120
	207O	209CA	4.341
40	209N	210N	3.152
	209N	212CG1	4.281
	209N	210CA	4.540
	209N	212N	4.914
	209CA	210N	2.421
45	209CA	210CA	3.796
	209CA	212CG1	4.372
	209CA	210C	4.462
	209CA	212N	4.662
	209CA	210CB	4.826
50	209CA	212CG2	4.959
	209CA	210CG	4.975
	209C	210CA	2.434
	209C	210C	3.026
	209C	210CB	3.693
55	209C	212N	3.800
	209C	210O	3.920
	209C	210CG	4.126
	209C	213N	4.177
	209C	210OD1	4.376
60	209C	212CG1	4.433
	209C	213CB	4.625
	209C	212CA	4.667
	209C	210ND2	4.747
	209C	212CG2	4.782
65	209C	212CB	4.818
	209C	212C	4.948
	209O	210N	2.245
	209O	210CA	2.777
	209O	210C	2.905
70	209O	213N	2.972
	209O	212N	3.043
	209O	210O	3.487
	209O	213CB	3.674
	209O	212CA	3.685
75	209O	212C	3.773
	209O	212CG2	3.827
	209O	213CA	3.906
	209O	212CG1	3.916
	209O	212CB	3.947
80	209O	210CB	4.249
	209O	210CG	4.878
	209O	212O	4.958
	209O	210OD1	4.993
	210N	212N	4.398

	210N	213N	4.866
	210N	213CB	4.979
	210CA	213CB	4.405
	210CA	212N	4.438
5	210CA	213N	4.596
	210C	213N	3.886
	210C	213CB	4.239
	210C	213CA	4.591
	210C	212CA	4.624
10	210C	212C	4.679
	210O	213N	3.501
	210O	213CB	3.633
	210O	212N	3.644
	210O	213CA	3.933
15	210O	213C	4.126
	210O	212C	4.352
	210O	212CA	4.631
	210O	213CG	4.674
	210O	213CD1	4.727
20	212N	213N	2.784
	212N	213CA	4.205
	212N	213C	4.773
	212N	213CB	4.895
	212CA	213N	2.468
25	212CA	213CA	3.845
	212CA	213C	4.420
	212CA	216N	4.888
	212CA	213CB	4.891
	212CB	213N	3.397
30	212CB	213CA	4.698
	212CB	304CE1	4.881
	212CG1	213N	4.408
	212CG2	213N	3.253
	212CG2	213CA	4.264
35	212CG2	304CE1	4.815
	212C	213N	1.335
	212C	213CA	2.440
	212C	213C	2.994
	212C	213CB	3.710
40	212C	213O	3.755
	212C	216N	3.855
	212C	216CB	4.183
	212C	216CA	4.658
	212C	213CG	4.966
45	212O	213N	2.245
	212O	216N	2.670
	212O	213CA	2.759
	212O	213C	2.887
	212O	216CB	3.134
50	212O	213O	3.255
	212O	216CA	3.457
	212O	213CB	4.240
	212O	304CE1	4.399
	212O	216C	4.660
55	212O	304CZ	4.701
	213N	216N	4.607
	213N	216CB	4.734
	213CA	250CD1	4.134
	213CA	216CB	4.363
60	213CA	216N	4.434
	213CA	250CG1	4.898
	213CA	216CA	4.958
	213CB	250CD1	4.585
	213CG	250CG1	4.288
65	213CG	250CD1	4.298
	213CG	249NH2	4.361
	213CD1	249NH2	4.189
	213CD1	250CG1	4.969
	213CD1	249CZ	4.976
70	213CD2	250CG1	3.388
	213CD2	250CD1	3.646
	213CD2	247ND2	4.070
	213CD2	247OD1	4.172
	213CD2	249NH2	4.297
75	213CD2	249CB	4.423
	213CD2	247CG	4.542
	213CD2	250CB	4.579
	213CD2	250N	4.650
	213CD2	249CD	4.689
80	213CD2	250CA	4.924
	213CE1	249NH2	3.969
	213CE1	249CZ	4.403
	213CE1	249NH1	4.789
	213CE1	250CG1	4.914

	213CE1	249NE	4.963
	213CE2	250CG1	3.302
	213CE2	249CB	3.341
	213CE2	250N	3.738
5	213CE2	213CB	3.785
	213CE2	247OD1	3.897
	213CE2	249C	4.015
	213CE2	249CD	4.057
	213CE2	249NH2	4.080
10	213CE2	250CD1	4.089
	213CE2	250CA	4.157
	213CE2	250CB	4.248
	213CE2	249CG	4.314
	213CE2	249CA	4.346
15	213CE2	249NE	4.420
	213CE2	249CZ	4.459
	213CE2	247ND2	4.468
	213CE2	249O	4.492
	213CE2	247CG	4.604
20	213CZ	249CB	3.765
	213CZ	249NH2	3.909
	213CZ	249CZ	4.112
	213CZ	250CG1	4.148
	213CZ	249NE	4.295
25	213CZ	249C	4.377
	213CZ	249CD	4.394
	213CZ	250N	4.428
	213CZ	249O	4.508
	213CZ	249NH1	4.708
30	213CZ	249CG	4.729
	213CZ	250CA	4.749
	213CZ	249CA	4.754
	213C	216N	3.701
	213C	216CB	4.305
35	213C	216CA	4.432
	213C	250CD1	4.614
	213C	216C	4.795
	213O	216N	3.163
	213O	212O	3.255
40	213O	213CB	3.375
	213O	213CG	3.382
	213O	213CD1	3.423
	213O	213N	3.471
	213O	216CA	3.662
45	213O	216CB	3.709
	213O	216C	3.731
	213O	250CD1	4.129
	213O	250CG1	4.545
	213O	216O	4.914
50	216N	304CZ	4.099
	216N	304CE2	4.314
	216N	304CE1	4.419
	216N	304CD2	4.807
	216N	304CD1	4.895
55	216CA	304CE2	3.847
	216CA	304CD2	3.926
	216CA	304CZ	3.934
	216CA	304CG	4.094
	216CA	304CE1	4.099
60	216CA	304CD1	4.169
	216CA	250CD1	4.669
	216CA	304CB	4.908
	216CA	220N	4.975
	216CA	220CD1	4.997
65	216CB	250CD1	3.531
	216CB	304CD1	3.550
	216CB	304CE1	3.746
	216CB	304CG	3.756
	216CB	304CZ	4.110
70	216CB	304CD2	4.118
	216CB	304CE2	4.288
	216CB	304CB	4.383
	216CB	250CG1	4.685
	216CB	220CD1	4.706
75	216C	220N	4.091
	216C	220CG	4.389
	216C	220CD1	4.410
	216C	220CB	4.425
	216C	250CD1	4.646
80	216C	304CD2	4.738
	216C	304CE2	4.889
	216C	220CA	4.901
	216C	304CG	4.985
	216O	220N	2.973

	216O	220CB	3.240
	216O	220CG	3.312
	216O	220CD1	3.600
	216O	220CA	3.682
5	216O	304CD2	4.466
	216O	220CD2	4.723
	216O	220C	4.729
	216O	304CG	4.812
	216O	304CE2	4.867
10	220CG	304CB	3.954
	220CG	304CD2	4.620
	220CG	304CG	4.655
	220CG	304N	4.839
	220CG	303C	4.910
15	220CG	304CA	4.914
	220CG	303O	4.942
	220CD1	250CG2	3.858
	220CD1	304CB	3.918
	220CD1	304N	4.769
20	220CD1	304CG	4.781
	220CD1	304CA	4.980
	220CD2	303O	3.794
	220CD2	303C	3.874
	220CD2	304CB	4.033
25	220CD2	303N	4.091
	220CD2	304N	4.170
	220CD2	303CA	4.361
	220CD2	304CA	4.531
	220CD2	304CD2	4.978
30	220CD2	304CG	4.997
	244N	303CA	4.590
	244N	303N	4.800
	244N	303CB	4.918
	244CA	246N	4.644
35	244CA	303CA	4.703
	244CA	303CB	4.756
	244CB	303CA	3.618
	244CB	303CB	3.663
	244CB	304N	4.380
40	244CB	303N	4.545
	244CB	303C	4.581
	244CB	246N	4.821
	244CG	303CB	4.261
	244CG	246N	4.437
45	244CG	303CA	4.495
	244CG	304O	4.536
	244CG	276O	4.605
	244CG	304N	4.694
	244CG	274OD1	4.945
50	244CD2	276O	3.276
	244CD2	276C	4.179
	244CD2	274OD1	4.296
	244CD2	276CA	4.491
	244CD2	276N	4.705
55	244CD2	303CB	4.764
	244ND1	246N	3.736
	244ND1	246CB	3.939
	244ND1	304O	4.002
	244ND1	246CA	4.065
60	244ND1	274OD1	4.288
	244ND1	274ND2	4.528
	244ND1	274CG	4.656
	244ND1	304N	4.729
	244CE1	274OD1	3.036
65	244CE1	274ND2	3.525
	244CE1	274CG	3.527
	244CE1	246N	4.024
	244CE1	246CA	4.116
	244CE1	246CB	4.253
70	244CE1	304O	4.409
	244CE1	276O	4.453
	244CE1	276CA	4.503
	244CE1	276N	4.607
	244CE1	274CB	4.806
75	244CE1	276C	4.903
	244NE2	274OD1	2.997
	244NE2	276O	3.189
	244NE2	276CA	3.620
	244NE2	276N	3.747
80	244NE2	276C	3.774
	244NE2	274CG	3.873
	244NE2	274ND2	4.248
	244NE2	246N	4.811
	244C	246N	3.311

	244C	246CA	4.639
	244O	246N	3.012
	244O	246CA	4.288
	244O	246CB	4.440
5	246N	247N	2.858
	246N	247CA	4.225
	246N	247O	4.519
	246N	274ND2	4.635
	246N	274CG	4.694
10	246N	247C	4.783
	246N	274CB	4.908
	246CA	247N	2.398
	246CA	274ND2	3.762
	246CA	247CA	3.780
15	246CA	274CG	4.159
	246CA	274CB	4.398
	246CA	247CB	4.470
	246CA	247ND2	4.518
	246CA	247O	4.632
20	246CA	247C	4.704
	246CA	274OD1	4.840
	246CA	247CG	4.866
	246CB	247N	3.017
	246CB	247ND2	3.981
25	246CB	274ND2	4.268
	246CB	247CA	4.360
	246CB	247CG	4.666
	246CB	247CB	4.733
	246CB	247O	4.816
30	246CB	250CG2	4.830
	246CB	250CD1	4.837
	246CB	250CB	4.978
	246CB	274CG	4.988
	246C	247CA	2.445
35	246C	247CB	3.093
	246C	247C	3.647
	246C	247ND2	3.730
	246C	247CG	3.789
	246C	247O	3.922
40	246C	274ND2	4.440
	246C	274CB	4.631
	246C	247OD1	4.815
	246C	274CG	4.829
	246O	247N	2.265
45	246O	246CA	2.400
	246O	247CA	2.826
	246O	247CB	3.054
	246O	247ND2	3.937
	246O	247CG	3.998
50	246O	274ND2	4.116
	246O	274CB	4.132
	246O	247C	4.279
	246O	274CG	4.548
	246O	247O	4.812
55	247CA	249N	4.491
	247CB	249N	4.494
	247CG	250N	3.957
	247CG	249N	4.038
	247CG	250CB	4.274
60	247CG	249CB	4.318
	247CG	250CG1	4.508
	247CG	249CA	4.619
	247CG	249CG	4.681
	247CG	250CD1	4.751
65	247CG	250CA	4.762
	247CG	249C	4.818
	247OD1	249N	3.007
	247OD1	250N	3.066
	247OD1	249CB	3.116
70	247OD1	249CA	3.431
	247OD1	249CG	3.604
	247OD1	249C	3.735
	247OD1	250CB	4.015
	247OD1	250CA	4.121
75	247OD1	249CD	4.172
	247OD1	250CG1	4.267
	247OD1	250CD1	4.870
	247OD1	249O	4.930
	247ND2	250CD1	3.820
80	247ND2	250CG1	3.953
	247ND2	250CB	4.004
	247ND2	250N	4.402
	247ND2	250CA	4.869
	247C	249N	3.305

	247C	250N	4.120
	247C	249CA	4.545
	247C	249C	4.779
	247C	250CB	4.900
5	247C	250CA	4.940
	247O	249N	3.360
	247O	250N	3.406
	247O	250CB	3.950
	247O	250CA	3.970
10	247O	250C	4.123
	247O	249C	4.217
	247O	249CA	4.373
	247O	250CG2	4.591
	249N	250N	2.817
15	249N	250CA	4.203
	249N	250C	4.666
	249CA	250N	2.413
	249CA	250CA	3.779
	249CA	250C	4.413
20	249CA	250CB	4.866
	249CB	250N	3.035
	249CB	250CA	4.353
	249CG	250N	4.416
	249C	250CA	2.410
25	249C	250C	3.035
	249C	250CB	3.720
	249C	250O	3.774
	249C	250CG1	4.278
	249C	250CG2	4.919
30	249O	250N	2.240
	249O	250CA	2.733
	249O	250C	3.038
	249O	250O	3.374
	249O	250CB	4.232
35	249O	250CG1	4.716
	274OD1	276CB	4.452
	274OD1	276C	4.632
	274OD1	276O	4.648
	274ND2	276N	4.935
40	274C	276N	3.322
	274C	276CA	4.688
	274O	276N	3.552
	274O	276CA	4.809
	303N	304CA	4.862
45	303CA	304N	2.430
	303CA	304CA	3.818
	303CA	304C	4.661
	303CA	304O	4.679
	303CA	304CB	4.684
50	303CB	304N	3.222
	303CB	304CA	4.521
	303CB	304O	4.818
	303CB	304C	4.963
	303C	304CA	2.452
55	303C	304CB	3.442
	303C	304C	3.504
	303C	304O	3.860
	303C	305N	4.467
	303C	304CG	4.748
60	303O	304N	2.247
	303O	304CA	2.806
	303O	304CB	3.762
	303O	304C	3.989
	303O	304O	4.630
65	303O	305N	4.674
	303O	304CG	4.827
	304N	305N	3.547
	304N	305CA	4.719
	304CA	305N	2.450
70	304CA	305CA	3.795
	304CA	305C	4.958
	304CB	305N	3.325
	304CG	305N	3.321
	304CG	305CA	4.469
75	304CG	305O	4.729
	304CD1	305N	3.304
	304CD1	305CA	4.052
	304CD1	305O	4.145
	304CD1	305C	4.383
80	304CD2	305N	4.139
	304CE1	305O	3.966
	304CE1	305N	4.100
	304CE1	305C	4.483
	304CE1	305CA	4.616

	304CE2	305N	4.804
	304CE2	305O	4.952
	304CZ	305O	4.401
	304CZ	305N	4.783
5	304C	305N	1.329
	304C	305CA	2.395
	304C	305C	3.718
	304C	305O	4.130
	304C	306N	4.754
10	304O	305N	2.239
	304O	305CA	2.696
	304O	305C	4.145
	304O	305O	4.826
	304O	306N	4.921
15	305N	306N	3.702
	305N	306CA	4.963
	305CA	306N	2.391
	305CA	306CA	3.771
	305CA	306O	4.400
20	305CA	306C	4.467
	305C	306CA	2.427
	305C	306C	3.071
	305C	306O	3.236
	305O	306N	2.248
25	305O	306CA	2.772
	305O	306C	2.996
	305O	306O	3.217
	306N	487CD2	4.792
	306CA	487CD2	4.048
30	306CA	487CG	4.502
	306CA	487CB	4.525
	306CA	487CE2	4.655
	306C	487CB	4.265
	306C	487CD2	4.576
35	306C	487CG	4.734
	306O	487CB	4.248
	306O	487CG	4.922

Table III provides the the atomic coordinates of the acetyl-CoA complex structure in the active site. Solvent molecules are omitted here for clarity, but can be found in Fig. 2. Residue 487 is Phe87 from the other monomer. Residue CAC is acetylated cysteine, and COA is the bound CoA molecule.

TABLE III

ATOM		RESIDUE		X	Y	Z	Occ	B
1	N	THR	28	32.909	0.319	26.935	1.00	14.64
2	CA	THR	28	31.524	0.759	27.053	1.00	16.73
3	CB	THR	28	31.399	2.311	26.861	1.00	18.66
4	OG1	THR	28	30.140	2.771	27.368	1.00	21.07
5	CG2	THR	28	31.523	2.702	25.394	1.00	14.87
6	C	THR	28	30.671	-0.021	26.041	1.00	15.95
7	O	THR	28	31.196	-0.755	25.190	1.00	14.39
8	N	TRP	32	24.685	1.112	27.156	1.00	18.61
9	CA	TRP	32	24.896	1.996	28.316	1.00	17.67
10	CB	TRP	32	26.253	1.657	28.999	1.00	18.46
11	CG	TRP	32	26.543	2.508	30.252	1.00	14.22
12	CD2	TRP	32	26.947	3.865	30.325	1.00	16.45
13	CE2	TRP	32	27.044	4.089	31.715	1.00	13.95
14	CE3	TRP	32	27.232	4.916	29.444	1.00	14.91
15	CD1	TRP	32	26.405	1.970	31.509	1.00	19.11
16	NE1	TRP	32	26.722	2.948	32.369	1.00	17.55
17	CZ2	TRP	32	27.417	5.348	32.222	1.00	16.49
18	CZ3	TRP	32	27.602	6.164	29.953	1.00	8.45
19	CH2	TRP	32	27.698	6.373	31.321	1.00	11.56
20	C	TRP	32	24.917	3.414	27.781	1.00	16.08
21	O	TRP	32	24.363	4.325	28.378	1.00	17.69
22	N	ILE	33	25.536	3.534	26.593	1.00	16.72
23	CA	ILE	33	25.591	4.911	26.052	1.00	17.89
24	CB	ILE	33	26.670	5.169	24.944	1.00	20.24
25	CG2	ILE	33	26.790	6.671	24.704	1.00	18.87
26	CG1	ILE	33	28.038	4.571	25.295	1.00	16.21
27	CD1	ILE	33	28.930	4.480	24.013	1.00	24.09
28	C	ILE	33	24.196	5.403	25.732	1.00	18.98
29	O	ILE	33	23.877	6.540	26.194	1.00	18.61
30	N	ARG	36	22.046	6.096	28.836	1.00	20.61
31	CA	ARG	36	22.587	7.077	29.780	1.00	20.93
32	CB	ARG	36	23.940	6.602	30.339	1.00	19.27
33	CG	ARG	36	23.882	5.328	31.146	1.00	20.40
34	CD	ARG	36	23.627	5.619	32.605	1.00	22.27
35	NE	ARG	36	23.511	4.396	33.393	1.00	27.02
36	CZ	ARG	36	23.867	4.298	34.670	1.00	25.93
37	NH1	ARG	36	23.734	3.152	35.315	1.00	26.63

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
38	NH2	ARG	36	24.330	5.355	35.318	1.00	23.35
39	C	ARG	36	22.702	8.517	29.247	1.00	18.28
40	O	ARG	36	22.703	9.462	30.029	1.00	17.41
41	N	THR	37	22.798	8.697	27.936	1.00	18.97
42	CA	THR	37	22.932	10.050	27.405	1.00	21.02
43	CB	THR	37	24.388	10.371	26.949	1.00	18.78
44	OG1	THR	37	24.793	9.461	25.925	1.00	17.72
45	CG2	THR	37	25.347	10.293	28.084	1.00	21.35
46	C	THR	37	22.048	10.362	26.222	1.00	20.16
47	O	THR	37	21.914	11.534	25.839	1.00	25.43
48	N	CAC	112	30.456	25.709	28.104	1.00	10.38
49	CA	CAC	112	29.270	25.229	27.412	1.00	14.44
50	CB	CAC	112	28.799	23.888	27.980	1.00	17.69
51	SG	CAC	112	29.712	22.439	27.254	1.00	17.65
52	CD	CAC	112	32.183	21.508	28.594	1.00	24.17
53	CE	CAC	112	30.937	22.403	28.616	1.00	21.28
54	OE	CAC	112	30.752	23.125	29.602	1.00	25.29
55	C	CAC	112	28.167	26.294	27.295	1.00	11.81
56	O	CAC	112	27.369	26.232	26.368	1.00	10.19
57	N	LEU	142	35.611	19.985	21.261	1.00	10.22
58	CA	LEU	142	35.860	19.347	22.539	1.00	13.06
59	CB	LEU	142	34.735	19.597	23.555	1.00	12.36
60	CG	LEU	142	34.583	20.999	24.171	1.00	11.62
61	CD1	LEU	142	33.937	20.919	25.543	1.00	5.06
62	CD2	LEU	142	35.940	21.651	24.300	1.00	10.88
63	C	LEU	142	36.175	17.851	22.433	1.00	13.55
64	O	LEU	142	36.786	17.299	23.322	1.00	19.07
65	N	ARG	151	36.295	6.724	29.164	1.00	23.03
66	CA	ARG	151	34.919	6.417	28.730	1.00	23.11
67	CB	ARG	151	34.470	5.004	29.175	1.00	16.86
68	CG	ARG	151	34.348	4.774	30.666	1.00	15.32
69	CD	ARG	151	33.926	3.335	30.928	1.00	7.13
70	NE	ARG	151	33.779	3.086	32.349	1.00	10.71
71	CZ	ARG	151	33.378	1.927	32.869	1.00	3.91
72	NH1	ARG	151	33.268	1.783	34.179	1.00	4.61
73	NH2	ARG	151	33.078	0.930	32.071	1.00	10.10
74	C	ARG	151	33.873	7.478	29.120	1.00	17.49
75	O	ARG	151	33.012	7.828	28.317	1.00	17.71
76	N	GLY	152	34.016	8.044	30.309	1.00	17.52
77	CA	GLY	152	33.070	9.045	30.776	1.00	16.37
78	C	GLY	152	33.062	10.401	30.082	1.00	15.84
79	O	GLY	152	32.246	11.248	30.439	1.00	21.56

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
80	N	ILE	155	32.443	9.844	25.187	1.00	7.71
81	CA	ILE	155	31.083	9.426	24.707	1.00	12.55
82	CB	ILE	155	30.385	8.425	25.708	1.00	11.77
83	CG2	ILE	155	31.197	7.148	25.866	1.00	11.90
84	CG1	ILE	155	30.158	9.085	27.088	1.00	12.15
85	CD1	ILE	155	29.158	8.276	27.966	1.00	11.79
86	C	ILE	155	30.193	10.622	24.373	1.00	10.55
87	O	ILE	155	29.530	10.593	23.314	1.00	14.21
88	N	ILE	156	30.115	11.601	25.228	1.00	15.15
89	CA	ILE	156	29.284	12.781	24.971	1.00	13.87
90	CB	ILE	156	28.912	13.460	26.383	1.00	18.45
91	CG2	ILE	156	27.632	12.860	26.931	1.00	23.09
92	CG1	ILE	156	30.082	13.252	27.370	1.00	15.34
93	CD1	ILE	156	29.617	12.611	28.714	1.00	19.30
94	C	ILE	156	29.845	13.826	24.026	1.00	13.98
95	O	ILE	156	29.049	14.365	23.211	1.00	9.76
96	N	PHE	157	31.114	14.104	24.000	1.00	10.77
97	CA	PHE	157	31.656	15.152	23.157	1.00	7.33
98	CB	PHE	157	32.859	15.790	23.759	1.00	4.54
99	CG	PHE	157	32.560	16.451	25.090	1.00	7.66
100	CD1	PHE	157	32.946	15.788	26.255	1.00	3.98
101	CD2	PHE	157	31.915	17.650	25.184	1.00	5.65
102	CE1	PHE	157	32.660	16.349	27.491	1.00	9.88
103	CE2	PHE	157	31.630	18.205	26.422	1.00	4.05
104	CZ	PHE	157	32.018	17.536	27.588	1.00	6.80
105	C	PHE	157	31.810	14.851	21.690	1.00	10.70
106	O	PHE	157	32.380	13.859	21.257	1.00	13.03
107	N	LEU	189	34.231	20.663	36.441	1.00	15.69
108	CA	LEU	189	34.309	20.542	34.989	1.00	15.11
109	CB	LEU	189	32.983	20.982	34.350	1.00	10.07
110	CG	LEU	189	32.807	20.922	32.844	1.00	7.51
111	CD1	LEU	189	33.311	19.593	32.263	1.00	10.35
112	CD2	LEU	189	31.343	21.142	32.523	1.00	7.61
113	C	LEU	189	35.464	21.418	34.538	1.00	15.40
114	O	LEU	189	35.452	22.612	34.812	1.00	16.51
115	N	LEU	205	40.306	17.390	29.143	1.00	13.16
116	CA	LEU	205	39.050	17.802	29.770	1.00	15.27
117	CB	LEU	205	37.963	17.874	28.694	1.00	12.62
118	CG	LEU	205	36.505	18.215	29.034	1.00	14.99
119	CD1	LEU	205	35.817	18.527	27.706	1.00	15.12
120	CD2	LEU	205	35.773	17.085	29.762	1.00	11.51
121	C	LEU	205	38.658	16.793	30.846	1.00	15.81

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
122	O	LEU	205	38.675	15.588	30.594	1.00	20.20
123	N	MET	207	35.792	15.888	34.121	1.00	18.42
124	CA	MET	207	34.419	16.232	34.463	1.00	16.18
125	CB	MET	207	33.555	16.227	33.174	1.00	17.87
126	CG	MET	207	32.024	16.237	33.467	1.00	17.17
127	SD	MET	207	30.990	16.464	32.044	2.00	17.60
128	CE	MET	207	31.340	14.797	31.582	1.00	22.99
129	C	MET	207	33.790	15.238	35.466	1.00	16.62
130	O	MET	207	33.726	14.046	35.222	1.00	18.22
131	N	GLY	209	30.811	14.103	36.169	1.00	12.42
132	CA	GLY	209	29.492	14.040	35.588	1.00	16.72
133	C	GLY	209	28.358	14.011	36.516	1.00	19.06
134	O	GLY	209	27.487	14.883	36.423	1.00	20.59
135	N	ASN	210	28.284	13.037	37.418	1.00	21.24
136	CA	ASN	210	27.150	13.010	38.362	1.00	24.44
137	CB	ASN	210	27.198	11.753	39.171	1.00	25.49
138	CG	ASN	210	27.160	11.958	40.631	1.00	33.50
139	OD1	ASN	210	26.177	11.619	41.309	1.00	34.80
140	ND2	ASN	210	28.217	12.429	41.247	1.00	32.41
141	C	ASN	210	26.970	14.201	39.196	1.00	25.55
142	O	ASN	210	25.858	14.799	39.270	1.00	27.11
143	N	VAL	212	27.967	17.255	38.323	1.00	18.86
144	CA	VAL	212	27.657	18.365	37.397	1.00	19.45
145	CB	VAL	212	28.483	18.363	36.115	1.00	13.66
146	CG1	VAL	212	28.142	19.417	35.091	1.00	10.49
147	CG2	VAL	212	29.921	18.642	36.480	1.00	11.31
148	C	VAL	212	26.176	18.359	36.977	1.00	25.20
149	O	VAL	212	25.455	19.359	36.929	1.00	27.15
150	N	PHE	213	25.738	17.114	36.763	1.00	24.63
151	CA	PHE	213	24.361	16.813	36.336	1.00	25.87
152	CB	PHE	213	24.203	15.287	36.398	1.00	23.74
153	CG	PHE	213	22.788	14.958	36.099	1.00	23.97
154	CD1	PHE	213	22.533	14.398	34.810	1.00	27.08
155	CD2	PHE	213	21.752	14.909	36.974	1.00	22.61
156	CE1	PHE	213	21.275	13.964	34.464	1.00	23.26
157	CE2	PHE	213	20.480	14.482	36.625	1.00	23.27
158	CZ	PHE	213	20.223	13.976	35.335	1.00	21.74
159	C	PHE	213	23.356	17.458	37.319	1.00	26.46
160	O	PHE	213	22.395	18.091	36.945	1.00	28.12
161	N	ALA	216	23.435	21.215	37.390	1.00	21.80
162	CA	ALA	216	22.949	21.675	36.100	1.00	19.74
163	CB	ALA	216	23.464	20.861	34.933	1.00	18.25

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
164	C	ALA	216	21.440	21.811	36.028	1.00	20.86
165	O	ALA	216	20.936	22.882	35.612	1.00	14.72
166	N	HIS	244	21.005	23.509	25.764	1.00	14.90
167	CA	HIS	244	22.348	23.098	25.390	1.00	17.43
168	CB	HIS	244	23.328	23.551	26.478	1.00	17.97
169	CG	HIS	244	24.644	22.836	26.459	1.00	18.58
170	CD2	HIS	244	25.582	22.714	25.488	1.00	18.43
171	ND1	HIS	244	25.123	22.136	27.546	1.00	18.75
172	CE1	HIS	244	26.295	21.608	27.243	1.00	21.88
173	NE2	HIS	244	26.597	21.944	26.000	1.00	17.34
174	C	HIS	244	22.190	21.563	25.366	1.00	17.94
175	O	HIS	244	21.579	20.979	26.286	1.00	18.08
176	N	ALA	246	23.569	18.461	26.118	1.00	19.92
177	CA	ALA	246	24.594	17.753	26.886	1.00	22.75
178	CB	ALA	246	24.851	18.474	28.207	1.00	20.40
179	C	ALA	246	24.197	16.301	27.174	1.00	25.65
180	O	ALA	246	24.941	15.364	26.869	1.00	27.18
181	N	ASN	247	23.035	16.122	27.793	1.00	26.14
182	CA	ASN	247	22.545	14.795	28.146	1.00	26.38
183	CB	ASN	247	22.964	14.464	29.587	1.00	28.11
184	CG	ASN	247	22.574	13.044	30.019	1.00	32.46
185	OD1	ASN	247	21.552	12.486	29.583	1.00	30.09
186	ND2	ASN	247	23.371	12.470	30.912	1.00	31.19
187	C	ASN	247	21.021	14.827	28.020	1.00	26.38
188	O	ASN	247	20.381	15.783	28.497	1.00	24.78
189	N	ARG	249	18.806	13.418	29.619	1.00	26.17
190	CA	ARG	249	18.082	13.368	30.918	1.00	30.19
191	CB	ARG	249	18.684	12.450	31.892	1.00	35.11
192	CG	ARG	249	20.149	12.514	32.084	1.00	40.00
193	CD	ARG	249	20.737	11.377	33.040	1.00	40.00
194	NE	ARG	249	19.770	10.270	32.981	1.00	40.00
195	CZ	ARG	249	20.131	8.991	32.720	1.00	40.00
196	NH1	ARG	249	19.206	8.005	32.728	1.00	40.00
197	NH2	ARG	249	21.400	8.728	32.469	1.00	40.00
198	C	ARG	249	17.883	14.720	31.505	1.00	30.27
199	O	ARG	249	16.848	15.128	31.999	1.00	29.21
200	N	ILE	250	19.022	15.485	31.317	1.00	31.13
201	CA	ILE	250	18.989	16.891	31.777	1.00	30.26
202	CB	ILE	250	20.417	17.557	31.646	1.00	31.49
203	CG2	ILE	250	20.269	19.060	31.848	1.00	27.57
204	CG1	ILE	250	21.391	16.967	32.703	1.00	27.31
205	CD1	ILE	250	22.804	17.587	32.626	1.00	29.25

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
206	C	ILE	250	17.878	17.652	31.051	1.00	29.32
207	O	ILE	250	17.014	18.274	31.667	1.00	30.29
208	N	ASN	274	27.325	16.399	22.555	1.00	13.47
209	CA	ASN	274	27.474	17.720	23.155	1.00	13.39
210	CB	ASN	274	27.818	17.530	24.622	1.00	15.56
211	CG	ASN	274	27.960	18.816	25.366	1.00	17.87
212	OD1	ASN	274	28.135	19.881	24.780	1.00	24.67
213	ND2	ASN	274	27.890	18.729	26.689	1.00	19.64
214	C	ASN	274	28.638	18.414	22.458	1.00	14.33
215	O	ASN	274	29.770	17.971	22.613	1.00	12.94
216	N	SER	276	29.549	21.633	22.863	1.00	7.51
217	CA	SER	276	29.823	22.861	23.613	1.00	13.37
218	CB	SER	276	31.354	23.045	23.758	1.00	16.08
219	OG	SER	276	31.709	24.178	24.552	1.00	13.44
220	C	SER	276	29.132	24.114	23.029	1.00	13.89
221	O	SER	276	27.945	24.062	22.700	1.00	11.72
222	N	PHE	304	24.334	25.567	30.088	1.00	14.66
223	CA	PHE	304	25.107	25.471	31.332	1.00	17.36
224	CB	PHE	304	24.396	24.476	32.274	1.00	14.19
225	CG	PHE	304	25.035	24.321	33.630	1.00	14.80
226	CD1	PHE	304	26.179	23.562	33.795	1.00	13.55
227	CD2	PHE	304	24.464	24.909	34.748	1.00	18.11
228	CE1	PHE	304	26.751	23.388	35.053	1.00	13.17
229	CE2	PHE	304	25.024	24.744	36.014	1.00	19.56
230	CZ	PHE	304	26.175	23.977	36.166	1.00	18.61
231	C	PHE	304	26.495	24.936	30.934	1.00	18.48
232	O	PHE	304	26.597	24.072	30.048	1.00	19.82
233	N	GLY	305	27.546	25.411	31.603	1.00	20.16
234	CA	GLY	305	28.889	24.966	31.272	1.00	18.15
235	C	GLY	305	29.950	25.008	32.367	1.00	15.06
236	O	GLY	305	29.701	25.407	33.507	1.00	11.78
237	N	GLY	306	31.145	24.556	31.988	1.00	16.84
238	CA	GLY	306	32.290	24.514	32.875	1.00	16.87
239	C	GLY	306	32.529	25.856	33.525	1.00	19.36
240	O	GLY	306	32.236	26.899	32.934	1.00	17.63
241	N	PHE	487	38.425	26.469	30.807	1.00	13.64
242	CA	PHE	487	37.277	26.474	31.704	1.00	12.94
243	CB	PHE	487	35.953	26.064	31.031	1.00	16.12
244	CG	PHE	487	35.967	24.728	30.332	1.00	10.50
245	CD1	PHE	487	36.055	24.668	28.952	1.00	11.96
246	CD2	PHE	487	35.776	23.548	31.043	1.00	14.59
247	CE1	PHE	487	35.943	23.450	28.275	1.00	11.67

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
248	CE2	PHE	487	35.665	22.321	30.373	1.00	10.83
249	CZ	PHE	487	35.748	22.283	28.989	1.00	9.41
250	C	PHE	487	37.606	25.574	32.861	1.00	13.75
251	O	PHE	487	38.217	24.529	32.661	1.00	18.53
252	AO6	COA	350	25.886	9.541	33.559	1.00	40.00
253	AP2	COA	350	25.938	8.466	34.779	1.00	40.00
254	AO4	COA	350	25.984	7.033	34.193	1.00	40.00
255	AO5	COA	350	24.688	8.689	35.674	1.00	40.00
256	AO3	COA	350	27.383	8.800	35.491	1.00	40.00
257	AP1	COA	350	27.959	7.998	36.780	1.00	40.00
258	AO1	COA	350	26.887	7.993	37.879	1.00	40.00
259	AO2	COA	350	29.237	8.653	37.296	1.00	40.00
260	AO5*	COA	350	28.201	6.460	36.164	1.00	40.00
261	AC5*	COA	350	27.718	5.279	36.817	1.00	39.18
262	AC4*	COA	350	28.472	4.019	36.378	1.00	37.65
263	AO4*	COA	350	28.702	4.012	34.931	1.00	35.45
264	AC3*	COA	350	29.898	3.856	36.965	1.00	37.54
265	AO3*	COA	350	30.205	2.474	37.178	1.00	40.00
266	AP3*	COA	350	31.518	2.029	38.160	1.00	40.00
267	AO7	COA	350	32.888	2.220	37.337	1.00	40.00
268	AO8	COA	350	31.503	3.018	39.420	1.00	40.00
269	AO9	COA	350	31.296	0.500	38.522	1.00	40.00
270	AC2*	COA	350	30.688	4.469	35.850	1.00	32.65
271	AO2*	COA	350	32.112	4.433	35.932	1.00	24.96
272	AC1*	COA	350	30.098	3.815	34.584	1.00	27.72
273	AN9	COA	350	30.429	4.564	33.382	1.00	20.99
274	AC8	COA	350	30.840	5.878	33.186	1.00	21.31
275	AN7	COA	350	30.992	6.002	31.788	1.00	18.53
276	AC5	COA	350	30.700	4.873	31.234	1.00	12.67
277	AC6	COA	350	30.698	4.501	29.898	1.00	12.21
278	AN6	COA	350	31.039	5.381	28.963	1.00	15.81
279	AN1	COA	350	30.338	3.249	29.672	1.00	17.72
280	AC2	COA	350	30.014	2.442	30.654	1.00	11.38
281	AN3	COA	350	29.997	2.743	31.973	1.00	15.08
282	AC4	COA	350	30.341	3.964	32.268	1.00	15.56
283	PS1	COA	350	27.926	20.647	30.314	1.00	40.00
284	PC2	COA	350	26.439	19.897	31.045	1.00	40.00
285	PC3	COA	350	26.760	18.654	31.835	1.00	40.00
286	PN4	COA	350	26.965	17.518	30.873	1.00	40.00
287	PC5	COA	350	27.350	16.338	31.273	1.00	40.00
288	PO5	COA	350	27.542	15.370	30.476	1.00	40.00
289	PC6	COA	350	27.580	16.199	32.745	1.00	40.00

Table III Cont.

ATOM		RESIDUE		X	Y	Z	Occ	B
290	PC7	COA	350	26.255	15.801	33.363	1.00	40.00
291	PN8	COA	350	26.292	14.370	33.634	1.00	40.00
292	PC9	COA	350	26.176	13.440	32.669	1.00	37.37
293	PO9	COA	350	25.948	13.691	31.437	1.00	31.87
294	PC10	COA	350	26.320	11.982	33.151	1.00	38.48
295	PO10	COA	350	26.849	11.940	34.496	1.00	37.07
296	PC11	COA	350	27.172	11.057	32.178	1.00	40.00
297	PC13	COA	350	28.667	11.476	32.189	1.00	40.00
298	PC14	COA	350	26.632	11.101	30.745	1.00	40.00
299	PC12	COA	350	26.933	9.588	32.579	1.00	40.00

Mutants and Derivatives

The invention further provides homologues, co-complexes and mutants of the *E. coli* FabH crystal structure of the invention.

The term "homologue" means a protein having at least 30% amino acid sequence identity with *E. coli* FabH or any of its functional domains.

The term "co-complex" means FabH or a mutant or homologue of FabH in covalent or non-covalent association with a chemical entity or compound.

The term "mutant" refers to a FabH polypeptide, i.e., a polypeptide displaying the biological activity of wild-type FabH activity, characterized by the replacement of at least one amino acid from the wild-type FabH sequence. Such a mutant may be prepared, for example, by expression of *E. coli* FabH cDNA previously altered in its coding sequence by oligonucleotide-directed mutagenesis.

E. coli FabH mutants may also be generated by site-specific incorporation of unnatural amino acids into the FabH proteins using the general biosynthetic method of C. J. Noren et al, Science, 244:182-188 (1989). In this method, the codon encoding the amino acid of interest in wild-type FabH enzyme is replaced by a "blank" nonsense codon, TAG, using oligonucleotide-directed mutagenesis. A suppressor tRNA directed against this codon is then chemically aminoacylated *in vitro* with the desired unnatural amino acid. The aminoacylated tRNA is then added to an *in vitro* translation system to yield a mutant FabH enzyme with the site-specific incorporated unnatural amino acid.

Selenomethionine may be incorporated into wild-type or mutant FabH by expression of *E. coli* FabH -encoding cDNAs in auxotrophic or non- auxotrophic *E. coli* strains [W. A. Hendrickson et al, EMBO J., 9(5):1665-1672 (1990)]. In this method, the wild-type or mutagenized FabH cDNA may be expressed in a host organism on a growth

medium depleted of either natural methionine but enriched in selenomethionine. The location(s) of the Se atom(s) can be determined by X-ray diffraction analysis at three or four different wavelengths. This information, in turn, is used to generate the phase information used to construct three-dimensional structure of the enzyme.

II. Methods of Identifying Inhibitors of the Novel FabH Crystalline Structure

Another aspect of this invention involves a method for identifying inhibitors of a FabH enzyme characterized by the crystal structure and novel active site described herein, and the inhibitors themselves. The novel *E. coli* FabH crystalline structure of the invention, or the structure of *E. coli* FabH homologue, permits the identification of inhibitors of FabH activity. Such inhibitors may be competitive, binding to all or a portion of the active site of FabH; or non-competitive and bind to and inhibit FabH whether or not it is bound to another chemical entity.

One design approach is to probe the FabH crystal of the invention with molecules composed of a variety of different chemical entities to determine optimal sites for interaction between candidate inhibitors and the enzyme. For example, high resolution X-ray diffraction data collected from crystals saturated with solvent allows the determination of where each type of solvent molecule sticks. Small molecules that bind tightly to those sites can then be designed and synthesized and tested for their FabH inhibitor activity [J. Travis, Science, 262:1374 (1993)].

This invention also enables the development of compounds that can isomerize to short-lived reaction intermediates in the chemical reaction of a substrate or other compound that binds to or with FabH. Thus, the time-dependent analysis of structural changes in FabH during its interaction with other molecules is permitted. The reaction intermediates of FabH can also be deduced from the reaction product in co-complex with FabH. Such information is useful to design improved analogues of known FabH inhibitors or to design novel classes of inhibitors based on the reaction intermediates of the enzyme and enzyme-inhibitor co-complex. This provides a novel route for designing FabH inhibitors with both high specificity and stability.

Another approach made possible by this invention, is to screen computationally small molecule data bases for chemical entities or compounds that can bind in whole, or in part, to the FabH enzyme. In this screening, the quality of fit of such entities or compounds to the binding site may be judged either by shape complementarity or by estimated interaction energy [E. C. Meng et al, J. Comp. Chem., 13:505-524 (1992)].

Because FabH may crystallize in more than one crystal form, the structure coordinates of FabH, or portions thereof, as provided by this invention are particularly useful to solve the structure of those other crystal forms of FabH. They may also be used to solve the structure of FabH mutant co-complexes, or of the crystalline form of any other protein with significant amino acid sequence homology to any functional domain of FabH.

One method that may be employed for this purpose is molecular replacement. In this method, the unknown crystal structure, whether it is another crystal form of FabH, a FabH mutant, a FabH co-complex, a FabH from a different bacterial species, or the crystal of some other protein with significant amino acid sequence homology to any domain of FabH, may be determined using the FabH structure coordinates of this invention as provided in Figure 1 and Tables I - III. This method will provide an accurate structural form for the unknown crystal more quickly and efficiently than attempting to determine such information *ab initio*.

Thus, the FabH structure provided herein permits the screening of known molecules and/or the designing of new molecules which bind to the structure, particularly at the active site or substrate binding site, via the use of computerized evaluation systems. For example, computer modeling systems are available in which the sequence of the FabH, and the FabH structure (i.e., the atomic coordinates, bond distances between atoms in the active site region, etc. as provided by Figures 1-2 and Tables I - III herein) may be input. Thus, a machine readable medium may be encoded with data representing the coordinates of Figures 1-2 and Tables I - III. The computer then generates structural details of the site into which a test compound should bind, thereby enabling the determination of the complementary structural details of said test compound.

More particularly, the design of compounds that bind to or inhibit FabH according to this invention generally involves consideration of two factors. First, the compound must be capable of physically and structurally associating with FabH. Non-covalent molecular interactions important in the association of FabH with its substrate include hydrogen bonding, van der Waals and hydrophobic interactions.

Second, the compound must be able to assume a conformation that allows it to associate with FabH. Although certain portions of the compound will not directly participate in this association with FabH, those portions may still influence the overall conformation of the molecule. This, in turn, may have a significant impact on potency. Such conformational requirements include the overall three-dimensional structure and orientation of the chemical entity or compound in relation to all or a portion of the binding

site, e.g., active site or substrate binding sites of FabH, or the spacing between functional groups of a compound comprising several chemical entities that directly interact with FabH.

The potential inhibitory or binding effect of a chemical compound on FabH may be analyzed prior to its actual synthesis and testing by the use of computer modelling techniques. If the theoretical structure of the given compound suggests insufficient interaction and association between it and FabH, synthesis and testing of the compound is obviated. However, if computer modelling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to FabH and inhibit using a suitable assay. In this manner, synthesis of inoperative compounds may be avoided.

An inhibitory or other binding compound of FabH may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with the individual binding pockets or other areas of FabH.

One skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with FabH and more particularly with the individual binding pockets of the FabH active site or accessory binding sites. This process may begin by visual inspection of, for example, the active site on the computer screen based on the FabH coordinates in Figures 1-2 and Tables I - III. Selected fragments or chemical entities may then be positioned in a variety of orientations, or docked, within a binding pocket of FabH. Docking may be accomplished using software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER.

Specialized computer programs may also assist in the process of selecting fragments or chemical entities. These include:

1. GRID [P. J. Goodford, "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", J. Med. Chem., 28:849-857 (1985)]. GRID is available from Oxford University, Oxford, UK.

2. MCSS [A. Miranker and M. Karplus, "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method", Proteins: Structure, Function and Genetics, 11:29-34 (1991)]. MCSS is available from Molecular Simulations, Burlington, MA.

3. AUTODOCK [D. S. Goodsell and A. J. Olsen, "Automated Docking of Substrates to Proteins by Simulated Annealing", Proteins: Structure, Function, and

Genetics, 8:195-202 (1990)]. AUTODOCK is available from Scripps Research Institute, La Jolla, CA.

4. DOCK [I. D. Kuntz et al, "A Geometric Approach to Macromolecule-Ligand Interactions", J. Mol. Biol., 161:269-288 (1982)]. DOCK is available from University of California, San Francisco, CA.

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound or inhibitor. Assembly may be proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure coordinates of FabH. This would be followed by manual model building using software such as Quanta or Sybyl.

Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include:

1. CAVEAT [P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in Molecular Recognition in Chemical and Biological Problems", Special Pub., Royal Chem. Soc. 78, pp. 182-196 (1989)]. CAVEAT is available from the University of California, Berkeley, CA.

2. 3D Database systems such as MACCS-3D (MDL Information Systems, San Leandro, CA). This area is reviewed in Y. C. Martin, "3D Database Searching in Drug Design", J. Med. Chem., 35:2145-2154 (1992).

3. HOOK (available from Molecular Simulations, Burlington, MA).

Instead of proceeding to build a FabH inhibitor in a step-wise fashion one fragment or chemical entity at a time as described above, inhibitory or other FabH binding compounds may be designed as a whole or "*de novo*" using either an empty active site or optionally including some portion(s) of a known inhibitor(s). These methods include:

1. LUDI [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6:61-78 (1992)]. LUDI is available from Biosym Technologies, San Diego, CA.

2. LEGEND [Y. Nishibata and A. Itai, Tetrahedron, 47:8985 (1991)]. LEGEND is available from Molecular Simulations, Burlington, MA.

3. LeapFrog (available from Tripos Associates, St. Louis, MO).

Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", J. Med. Chem., 33:883-894 (1990). See also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", Current Opinions in

Structural Biology, 2:202-210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the structure of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P.S. Farmer, Drug Design, Ariens, E.J., ed., Vol. 10, pp 119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; C. Verlinde, Curr. Biol., 2:577-587 (1994); and I. D. Kuntz, Science, 257:1078-1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may be quickly and easily examined and expensive and lengthy testing avoided. Moreover, the need for actual synthesis of many compounds is effectively eliminated.

Once identified by the modelling techniques, the FabH inhibitor may be tested for bioactivity using standard techniques. For example, structure of the invention may be used in binding assays using conventional formats to screen inhibitors. One particularly suitable assay format includes the enzyme-linked immunosorbent assay (ELISA). Other assay formats may be used; these assay formats are not a limitation on the present invention.

In another aspect, the FabH structure of the invention permit the design and identification of synthetic compounds and/or other molecules which are characterized by the conformation of FabH of the invention. Using known computer systems, the coordinates of the FabH structure of the invention may be provided in machine readable form, the test compounds designed and/or screened and their conformations superimposed on the structure of the FabH of the invention. Subsequently, suitable candidates identified as above may be screened for the desired FabH inhibitory bioactivity, stability, and the like.

Once identified and screened for biological activity, these inhibitors may be used therapeutically or prophylactically to block FabH activity, and thus, bacterial replication.

The following examples illustrate various aspects of this invention. These examples do not limit the scope of this invention which is defined by the appended claims.

Example 1 - The Expression of FabH from *Escherichia coli* in *Escherichia coli*.

The strategy for the expression of the FabH from *Escherichia coli*, using *Escherichia coli* as a host was based on the PCR amplification of the *fabH* gene and the introduction of suitable restriction sites that allowed the cloning of the *fabH*-containing DNA fragment in the pET29 expression vector. After the PCR amplification the insert of the resultant recombinant plasmid, (pET29c hereafter), was sequenced to verify the absence

of artifacts introduced by the *Taq* polymerase reaction. Expression was monitored by SDS-polyacrylamide gel analysis.

A. Bacterial strains, Plasmids and Medium

The *Escherichia coli* strains used were: MAXEfficiency DH10B Competent Cells Genotype: F⁻ *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80d*lacZ*ΔM15 Δ*lacX*74 *deoR recA*1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ⁻ *rps*L *nup*G. *E. coli* cells were grown at 37°C in Luria Bertani broth (LB). These strains may all be obtained from commercial sources. BL21(DE3) competent cells for protein expression purchased by Novagen. The protocol used to make them electroporation-competent was the one provided by Invitrogen.

The plasmid used was pET29 [Novagen]. A detailed description of pET29 is provided in Figure 2. Briefly, pET29 is an expression vector of *E.coli* which is based on the T7 promoter-driven system and allows the selection of the recombinant clones by kanamycin resistance.

LB Medium. Per liter:

Bacto-tryptone	10 g
Bacto-yeast extract	5 g
NaCl	5 g

For plasmid propagation 0.1mg/ml of kanamycin was added to the medium.

B. DNA manipulations

Plasmid DNA was prepared by the rapid alkaline method (Sambrook et al, 1989). Transformations of *E. coli* cells were carried out according to the protocol provided with the DH10B or the electroporation method. The plasmids for sequencing were purified using QIAGEN mini-prep kit [QIAGEN]. DNA sequencing was carried out on supercoiled plasmid DNA by the dideoxy chain-termination method using the Thermo Sequenase cycle sequencing kit [ABI, BigDye, Applied BioSystems, USA]. Synthetic oligonucleotides [ordered in-house] were used as primers. Restriction enzymes and T4 DNA ligase were obtained from Gibco BRL (Life Technologies) and the experiments were carried out following the instructions provided by the suppliers.

The *fabH* gene from *E.coli* cloned in the pET29 vector was amplified by PCR using the primers:

(5'-TATACATATGTATACGAAGATTATTGGT-3'; SEQ ID NO:2) and:

(5'-ATATGGATCCCTAGAAACGAACCAGCGCGG-3'; SEQ ID NO:3).

NdeI and *BamHI* restriction sites were incorporated at the 5' and 3' ends respectively of each primer to facilitate ligation of the amplified DNA into vectors. Plasmid DNA (480 ng) was amplified in 100 ul of PCR mixture containing 200 uM deoxynucleotide triphosphates (dNTPs), 0.20 mM oligonucleotide primers, the manufacturer's buffer and 2.5 U of *pfu* (Stratagene). The following cycling parameters were used:

94°C 4 min

94°C 1 min, 55°C 40 sec, 72°C 1 min (30 cycles)

72°C 2 min

4°C

Polymerase chain reaction (PCR) was performed using the GeneAmp, PCR System 2400 [Perkin Elmer Cetus Co]. PCR-amplified DNA fragments were purified using Qiaquick PCR Purification kit for Rapid Purification of DNA Fragments [Qiagen].

C. Cloning of the *fabH* gene of *E. coli* in the expression vector pET29 of *E. coli*.

The cloning strategy is shown in Figure 2. PCR amplification of the *fabH* gene from *E. coli* using the primers AKK2 and AKK3 resulted in a DNA fragment of about 960 bp. This fragment was purified with Qiaquick PCR purification kit protocol (Qiagen) digested with *NdeI and BamHI*, purified, ligated overnight to the *NdeI and BamHI* sites of already digested vector pET29 to obtain the recombinant plasmid pET29c. The ligation mix was used to transform *E. coli* DH10B competent cells. The construction of pET29c was initially confirmed by restriction analysis of the plasmid purified from the transformants. The amplification with *Taq* DNA polymerase made the sequencing of the *fabH* of pET29c an obligatory step to confirm that no changes were introduced due to the low fidelity of this enzyme. Sequence analysis was accomplished by using T7 promoter and terminator primers. The sequencing of both strands showed that no artifacts had been introduced during the amplification process.

D. Small-scale production of FabH from *E. coli* in *E. coli*

The plasmid pET29c and the negative control pET29 (vector without insert) were used to transform the *E. coli* BL21(DE3) host strain. Single clones of BL21(DE3): pET29c cells were grown overnight at 37°C in 2 ml of LB medium in the presence of 0.1 mg/ml kanamycin. The cells were then diluted 100-fold in 10 ml LB with kanamycin. When the cultures reached a value of 0.5 at OD_{600} the *fabH* expression was induced by addition of isopropyl-thio-galactoside (IPTG) at 0.5 mM of final concentration.

After this induction 2 ml samples were taken at different times (1 and 2 hours). The cells were harvested in a microfuge for 3 min, the pellets were washed with 20 mM Tris-HCl pH 8, 1mM PMSF and resuspended in 100 ul of SDS-PAGE gel-loading buffer. The cells were broken by sonication (15 seconds). The samples were then boiled for 10 minutes and after one spin, 10 ml fractions were analyzed by SDS-PAGE according to the methods of Laemmli [U. K. Laemmli, Nature 227, 680-685 (1970)]. The 4-12% polyacrylamide gels [NOVEX] were stained with Coomassie blue. As shown in Figure 3 good expression levels were detected from the early stages after induction with IPTG. The evidence was the presence of a prominent band (lanes *2, 4 and 6* in Figure 3) that was in good agreement with the M_r predicted from the primary sequence. The FabH protein has a theoretical molecular weight of about 33,514 Da.

Example 2 - Large Scale Growth and Purification of FabH

A. Large Scale Growth

A 4 liter fermentation of *E.coli* BL21(DE3): pET29c was carried out in Luria Bertani medium (LB), containing 100 ug/ml kanamycin. The 8x500 ml flasks were inoculated at 1% (v/v) from an overnight secondary seed culture in single strength LB medium, containing 100 mg/ml kanamycin. The flasks were incubated at 37°C, agitated at 250 rpm in a benchtop shaker. The OD at 600 nm was monitored, and at 0.5 absorbance units, FabH expression was induced with the addition of isopropyl-thiogalactosidase to 0.5 mM and the cells harvested by centrifugation in a Sorval CSA rotor, 2 hours post induction. A total of 20 grams of cell paste was recovered.

LB Medium, per liter, contains the following components. The medium was supplied by the in-house laboratory.

Single strength

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g

B. Purification

1) Lysis

12.5 g of cells of *E. coli* overexpressing *E. coli* FabH obtained as described above, were resuspended in 140 ml of 20 mM Tris pH 7.9, 50 mM NaCl, 1mM EDTA, 1mM DTT, 10% glycerol, 1 mM PMSF (buffer A). Lysozyme (Sigma Chemicals: hen egg) was added to a final concentration of 1 mg/ml. Cells were incubated at 37°C for 20min. The cells were then lysed by sonication in an ice water bath (4x30sec). DNAse

(Sigma; bovine pancreas type 1) was added along with $MgCl_2$ and held at 37°C for 5 minutes. The solution was centrifuged in a Beckman JA-HS centrifuge at 14,000 g for 60 minutes using a Beckman JA-14 rotor.

2) Anion exchange

All chromatography was performed on a Pharmacia chromatography system, fitted with a UV detector (Pharmacia, Monitor UV-1). UV (at 280 nm) was monitored during all operations. All operations were performed at 4°C.

The supernatant from 1) was loaded onto a Q-Sepharose high performance (Pharmacia) column of 50 ml packed into a Pharmacia XK-26 column. The column was equilibrated with buffer A prior to loading. The column was then washed with buffer A (250 ml) at 4 ml/min, and eluted with a linear gradient of buffer A to 1M NaCl in buffer A over 80 minutes at 4 ml/min. The eluate was fractionated into 8 ml fractions using a Pharmacia FRAC 200.

The eluted fractions were assayed for FabH activity by measurement of incorporation of [^{14}C]Acetyl-CoA to Malonyl-ACP and , and for protein by the Bradford method. Active fractions were analyzed by reducing SDS-PAGE (NOVEX, NuPAGE Bis-Tris 4-12 % gradient gel). Active fractions pooled together and dialyzed against Buffer A.

3) Anion Exchange chromatography

The dialyzed material was loaded onto a MonoQ column equilibrated with buffer A (Pharmacia, 5/5). The column was washed with 20 ml of the equilibration buffer until 280 nm absorbance returned to base line and then eluted with a linear gradient of equilibration buffer to buffer A over 90 minutes at 0.5 ml/min. Fractions were pooled together, collected, assayed for FabH activity.

4) Hydroxyapatite/ buffer exchange

Eluted fractions are collected (1 ml fraction) and assayed for FabH activity and protein. Active fractions are pooled and the volume was doubled with Buffer B [20 mM Tris-HCl pH 7.4, 50 mM NaCl, 1 mM DTT and 10% glycerol] to reduce the salt concentration in half. The active eluate was loaded in a hydroxyapatite column and eluted with 0.5 M Potassium Phosphate pH 7.4. The active enzyme was buffer exchanged with 20 mM Tris pH 7.4, 50 mM NaCl 2 mM DTT. This product was greater than 97% purity by SDS PAGE and the activity showed an overall process yield of 60 % from 1). N-terminal amino acid analysis confirmed identity.

Example 3 - Fermentation, Purification and Characterization of seleno-methionine derivative of *Escherichia coli* FabH

A. Fermentation

To obtain soluble selmet-FabH for purification and crystallization studies, E. coli strain BL21 (DE3) was transformed with pET29c FabH. 50 ul of the seed culture expressing FabH gene product was inoculated into 100 ml of Luria broth, containing kanamycin (50 ug/ml) and glucose (0.6%). On reaching target density of 2 OD, the cells from the seed culture were isolated by centrifugation, resuspended in 100 ml of M9 minimal medium containing 1 mM $CaCl_2$, 1 mM $MgSO_4$, kanamycin (50 ug/ml) and glucose (0.6% w/v). The resuspended pellets were then added to 900 ml of the same medium and the cells were grown at 37 C to mid-log phase, A_{600} of 1.5, at which point lysine, phenylalanine, threonine at 100 mg/l each, and selenomethionine, isoleucine, leucine, and valine at 50 mg/l were added. The culture was shaken for 15 minutes, and then induced with 0.5mM isopropyl b-D-thiogalactopyranoside (IPTG). The culture was grown for 13 hours, and harvested by centrifugation (speed). 5 ml aliquots were taken prior to and during induction to monitor the expression of selenomethionine FabH. 12g of cell paste (wet wt) was recovered from 5L. In addition, to compare the expression of selenomethionine derivative to that of wt FabH, a one l culture was prepared under identical conditions except that the cells were grown in LB media.

B. Purification

All lysis and purification steps were carried out using degassed buffers in a cold room or on ice. 4.5 g of E. coli cells over expressing Fab H were resuspended in 50 ml of 20 mM Tris, 50 mM sodium chloride, 10% glycerol, 0.2 mM PMSF, 2 mM DTT, pH 7.9 (buffer A). Cells were lysed twice at 10,000 psi using Microfluidizer (Microfluidics Corporation, MA). Cell debris was removed by centrifugation (Sorvall RC-5B) at 35,000 g for 30 min. The supernatant was applied to a 2.6 x 4 cm Source Q column (Pharmacia) equilibrated in buffer A. The column was washed with 10 column volumes of buffer A, and eluted with a 10 column volume gradient of 0 to 1.0 M NaCl in buffer A. Eluted fractions were analyzed by 10% SDS-PAGE under reducing conditions. Fab H eluted at 0.2 - 0.25 M NaCl. Fab H containing fractions were pooled and applied to a 2.6 x 6 cm Hydroxyapatite column (Bio-Rad, Type I, 40u) equilibrated in buffer A. The column was eluted with a 30 column volume linear gradient of buffer A to 400 mM potassium phosphate, 10% glycerol, 2 mM DTT. Fab H, which eluted at 80-180 mM potassium phosphate, was diluted 1:2 with 50 mM Tris, 200 mM NaCl, 10% glycerol, 2 mM DTT, pH 7.5 (buffer B) and applied to a 1.6 X 7.5

cm Blue Sepharose column (Pharmacia) equilibrated in buffer B. The column was eluted with buffer B containing 1 M NaCl. Blue Sepharose eluted Fab H fractions were next applied to a 2.6 x 60 cm Superdex 200 size exclusion column (Pharmacia) equilibrated in 20 mM Tris, 50 mM NaCl, 2 mM DTT, pH 7.4. A total of 23 mg of Fab H was recovered which was concentrated to13 mg/ml using Amicon 3 filtration device.

C. Characterization

i). Mass Spectoscopy

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) data were obtained on a PerSeptive Biosystems Voyager RP laser desorption time-of-flight mass spectrometer. Protein samples were prepared for analysis by diluting analyte 1:5 with 3,5-Dimethoxy-4-hydroxy-cinnamic acid (10mg/ml in 2:1 0.1% trifluoroacetic acid/ acetonitrile) for a final concentration of 1-10 pmol/ul. Bovine Beta lactoglobulin A (Sigma) was included as an internal calibrant (MH^+ 18364 Da). Desorption/ionization was accomplished using photon irradiation from a 337-nm pulsed nitrogen laser and 30-keV accelerating energy. Spectra were averaged over ca. 100 laser scans.

The predicted molecular mass for native FabH is 33516 Da. MALDI-MS analysis of the selenomethionyl incoporated FabH protein construct provided a mass of 33,889 Da. This is in close agreement with the predicted +375Da shift in mass expected for the sulfur to selenium side-chain substitution of eight methionine residues within the protein (33,891 Da theoretical).

ii). N-terminal sequence analysis

Sequence analysis was performed on a Hewlett-Packard G1000A N-terminal Protein Sequencer with on-line PTH identification using an HP1100 HPLC. Samples were applied directly to biphasic sequencing cartridges and standard 3.1 sequencing method cycles were used.

N-terminal sequencing results showed negligible native methionine in the first residue. Instead, a unique PTH (phenylthiohydantoin) derivative was observed which eluted 1.6 minutes later than PTH-methionine, and did not coelute with any natural PTH-amino acid derivatives. The increase in hydrophobicity is consistent with the direct detection of the PTH-selenomethionyl amino acid derivative.

D. Measurement of ß-ketoacyl-ACP synthase III activity.

The enzyme catalyses the condensation of acetyl-CoA with malonyl-ACP to form acetoacetyl-ACP. The reaction can be described by three distinct steps: (a) the acyl group

of acetyl-CoA is transferred to the active site cysteine resulting in a acyl-enzyme thioester; (b) carbanion formation by the decarboxylation of malonyl-ACP; and (c) carbon-carbon bond formation by nucleophillic attack of the carbanion onto the carbonyl carbon atom of the acyl-enzyme thioester to yield the acetoacetyl-ACP product.

This reaction can be assayed in order to characterize the enzyme or identify specific inhibitors of its activity in two ways:

(1) Radiolabeled acetoacetyl-ACP formation can be specifically determined using [3H]-acetyl-CoA and malonyl-ACP. The [3H]-acetyl-CoA substrate is soluble in 10%TCA while the resulting [3H]-acetoacetyl-ACP is not. A reaction mixture containing 100mM sodium phosphate buffer pH7.0, 1mM DTT, 34uM acetyl-CoA, 0.15uM [3H]-acetyl-CoA (specific activity 25Ci/mmol), and 7uM malonyl-ACP is prepared and transfered to a microtiter plate with or without inhibitors already added. The enzyme is added last to start the reaction and the plate is incubated at 37 degrees C. Ten percent TCA is added to stop the reaction, and then 50ug of BSA as a carrier. Stopped reactions are filtered and washed 2 times with 10% TCA on Wallac GF/A filtermats using a TomTec harvester. The filtermats are dried at 60 degrees C and the radioactivity quantified using Wallac Betaplate scintillation cocktail and a Wallac Microbeta 1450 liquid scintillation counter. IC50s are generated using Grafit 4.0 software and solved using the equation $v = Vmax/(1 + I/IC50)$.

(2) FabG coupling can also be used to measure FabH production of acetoacetyl-ACP by following NADPH consumption at 340nm. FabG (ß-ketoacyl-ACP reductase) will specifically reduce the C3 carbonyl of acetoacetyl-ACP to form ß-hydroxybutyryl-ACP. This reduction requires the conversion of NADPH to form NADP+ which can be monitored by following the optical density at wavelength 340nm.

(3) FabD coupling is an available assay option in the absence of purified malonyl-ACP. FabD (Malonyl-CoA:ACP transacylase) is responsible for malonic acid transfer from malonyl-CoA to ACP to form malonyl-ACP. This activity can be exploited by applying the techniques described in (1) above together with *de novo* malonyl-ACP from the FabD reaction.

E. Ligand binding to FabH.

It is also possible to define ligand interactions with FabH in experiments that are not dependent upon enzyme catalyzed turnover of substrates. This type of experiment can be done in a number of ways:

(1) Effects of ligand binding upon enzyme intrinsic fluorescence (e.g. of tryptophan). Binding of either natural ligands or inhibitors may result in enzyme conformational changes which alter enzyme fluorescence. Using stopped-flow fluorescence equipment, this can be used to define the microscopic rate constants that describe binding. Alternatively, steady-state fluorescence titration methods can yield the overall dissociation constant for binding in the same way that these are accessed through enzyme inhibition experiments.

(2) Spectral effects of ligands. Where the ligands themselves are either fluorescent or possess chromophores that overlap with enzyme tryptophan fluorescence, binding can be detected either via changes in the ligand fluorescence properties (e.g. intensity, lifetime or polarization) or fluorescence resonance energy transfer with enzyme tryptophans. The ligands could either be inhibitors or variants of the natural ligands.

(3) Thermal analysis of the enzyme:ligand complex. Using calorimetric techniques (e.g. Isothermal Calorimetry, Differential Scanning Calorimetry) it is possible to detect thermal changes, or shifts in the stability of FabH which reports and therefore allows the characterization of ligand binding.

Example 3 - Crystallization of *E. coli* wild-type and selenomethionine mutant of FabH

A. Crystallization

All crystals were grown at room temperature using the sitting-drop vapor diffusion method. The drop solution was always a 1:1 mixture of the protein sample and the well solutions. For the crystal form 1 of the wild-type protein, the well solution contained 0.1 M HEPES buffer at pH 7.5 and 20% PEG8000. For the crystal form 2 of the selenomethionine mutant protein in complex with acetyl-CoA, the well solution contained 0.05 M Bis-Tris propane buffer at pH 7.0, 0.1 M $MgCl_2$ and 14% PEG6000. Crystals grew overnight and are approximately 0.1 to 0.2 mm in sizes.

B. X-ray Diffraction Characterization

All crystals were frozen in liquid nitrogen streams before their characterization using synchrotron X-ray radiation. Diffraction data for the apo form 1 crystal was collected to 2.0 Å resolution. The data is 97.1 % complete and 6 fold redundant with a merging R-

factor of 7.7 %. The crystal belongs to the orthorhombic spacegroup $P2_12_12_1$, with cell dimensions a = 63.1, b = 65.1 and c = 166.5 Å. For the Se-Met protein in complex with acetyl-CoA, data were collected at three different wavelengths: 0.9789, 0.9785 and 0.9414 Å. The three data set were of nearly identical quality, with about 80% completion, 6-fold redundancy, 8.5 %

merging R-factor, and 1.9 Å resolution. The form 2 crystal belongs to the tetragonal spacegroup $P4_12_12$, with a = b = 72. 4 and c = 102.8 Å.

C. Structure Solution

The crystal structure of the Se-Met *E. coli* FabH mutant in complex with acetyl-CoA was solved to 1.9 Å resolution using the MAD phasing technique with the data sets collected at three different wavelengths and the program SOLVE (Terwilliger & Berendzen, 1999, *Acta Cryst.* D55, 849-861). All eight Se-Met were located by SOLVE. The overall MAD phasing figure of merit was 0.6 to 1.9 Å resolution, and the overall Z score was as high as 148. The resulting electron density map was of very high quality. The structure of the apo enzyme (crystal form 1) was solved with the molecular replacement method using the acetyl-CoA complex structure as the search model. This crystal form had a FabH dimer in the asymmetric unit, and the R-factor of the solution was only 33%. Two-fold averaged map was then calculated and used for model building.

D. Model Building and Refinement

The electron density for the acetyl-CoA complex was very clear and a structure model for the whole FabH protein, the bound acetyl group and CoA, as well as 98 solvent molecules were built in the first round. Standard structural refinement protocols and manual model building led to the current model, which has an R-factor of 27 % to 1.9 Å resolution. The model for the apo FabH structure was also built readily, and refined to an R-factor of 18.9 % (R_{free} of 24.4%) to 2.0 Å resolution. Both models have excellent geometry and do not have any outliers in the Ramanchandran plot, indicating high quality of the atomic coordinates, which contain an estimated error of less than 0.3 Å.

This invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims. The disclosures of the patents, patent applications and publications cited herein are incorporated by reference in their entireties.